# TOXICOLOGICAL PROFILE FOR RDX

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

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# DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

# UPDATE STATEMENT

A draft for public comment Toxicological Profile for RDX was released in 2010. 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:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine/Applied Toxicology Branch
1600 Clifton Road NE
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Atlanta, Georgia 30333

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# FOREWORD

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

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a 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; more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information. 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.

Christopher J. Portier, Ph.D.
Assistant Administrator
Agency for Toxic Substances and Disease Registry

# QUICK REFERENCE FOR HEALTH CARE PROVIDERS

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

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***Primary Chapters/Sections of Interest***

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

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

**Chapter 3: Health Effects**: Specific health effects of a given hazardous compound are reported by 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**

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***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.

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***Other Agencies and Organizations***

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

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

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

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***Referrals***

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

# PEER REVIEW

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

1. Ping Gong, Ph.D., Senior Scientist, SpecPro, Inc., Vicksburg, Mississippi;

2. Sam Kacew, Ph.D., Associate Director of Toxicology, McLauglin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, Ontario; and

3. Sharon A. Meyer, Ph.D., Associate Professor, Department of Toxicology, College of Health Sciences, University of Louisiana at Monroe, Monroe, Louisiana.

These experts collectively have knowledge of RDX'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

This public health statement tells you about hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and the effects of exposure to it.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. RDX has been found in at least 31 of the 1,699 current or former NPL sites. Although the total number of NPL sites evaluated for this substance is not known, the possibility exists that the number of sites at which RDX is found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to this substance may be harmful.

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

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

## 1.1 WHAT IS RDX?

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| **Other names for RDX** | RDX stands for Royal Demolition Explosive, also known as cyclonite, hexogen, and hexahydro-1,3,5-trinitro-1,3,5-triazine. |
| **White crystalline solid** | RDX is an explosive on its own, and can be combined with other ingredients to make plastic explosives (C-4 contains 91% RDX). Its odor and taste are unknown. When heated, acrid fumes may be released. |

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| **Used in explosives** | RDX is used as an explosive. It is a synthetic product that does not occur naturally in the environment. |

## 1.2 WHAT HAPPENS TO RDX WHEN IT ENTERS THE ENVIRONMENT?

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| **Found in water, soil, and air** | RDX particles can enter air when it is disposed of by burning. RDX can enter water from disposal of waste water from ammunition plants. RDX can enter water or soil from spills or leaks from improper disposal at plants or hazardous waste sites and at current and former military installations. |
| **Removal from soil, water, and air** | RDX is slow dissolving in water. It does not bind significantly to soils and can leach to groundwater from soil. In water and air, RDX can break down in hours, but breaks down more slowly in soil. It does not build up in fish or people. |

## 1.3 HOW MIGHT I BE EXPOSED TO RDX?

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| **Air** | You may be exposed to RDX by the inhalation, oral, or dermal routes, or by any combination of these routes. Typically, only people who work with RDX can potentially breathe RDX dust or get it on their skin. You can be exposed if you breathe RDX fumes from explosions or bombing ranges of burning RDX. |
| **Water and soil** | You may be exposed to RDX by drinking contaminated water or by touching contaminated soil if you live near facilities that produce or use RDX. RDX has been found in water and soil near some ammunition plants, current or former military installations and storage areas. |
| **Food** | You may be exposed to RDX by ingesting agricultural crops grown in contaminated soils irrigated with contaminated water. |

## 1.4 HOW CAN RDX ENTER AND LEAVE MY BODY?

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| **Enter the body**<br>**• Inhalation**<br><br>**• Oral**<br><br>**• Dermal contact** | RDX can enter your body if you breathe in fumes of burning RDX or from the detonation of munitions containing RDX or if you breath in the dust from powdered RDX.<br><br>It can also enter the body if you drink water contaminated with RDX or accidentally or intentionally ingest explosives containing RDX.<br><br>Much less RDX can enter the body through the skin if you come in contact with dusts of RDX or with liquids containing RDX. |
| **Leave your body** | Based on observations made in humans and results from studies in animals, most of the RDX appears to be broken down rapidly in the body. These products, as well as unchanged RDX, are eliminated in the urine and exhaled air in a few days. RDX is not expected to accumulate in the body. |

## 1.5 HOW CAN RDX AFFECT MY HEALTH?

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| **Humans** | If you breathe in dusts of RDX or intentionally or accidentally swallow large amounts of RDX, you may develop seizures. The seizures are temporary and will stop after the RDX is eliminated from your body.<br><br>Some people exposed to large amounts of RDX also have alterations in blood pressure and in some components of the blood, but these effects may be secondary to the seizures.<br><br>We do not know the effects of long-term, low-level exposure to RDX. |
| **Laboratory animals** | Animals that had large amounts of RDX placed in the stomach with a tube or that ate food mixed with RDX for longer periods of time suffered seizures.<br><br>Rats and mice that ate RDX for 3 months or longer had decreased body weights and slight liver and kidney damage. |
| **Cancer** | There are no studies reported of cancer in people exposed to RDX.<br><br>The EPA has determined that RDX is a possible human carcinogen based on the presence of liver tumors in mice that were exposed to RDX in the food for 1–2 years. |

## 1.6 HOW CAN RDX AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

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| **Effects in children** | There are no studies of children exposed to RDX, but a child who accidentally ingested RDX had seizures, which is the same effect that occurs in adults exposed to high amounts of RDX.<br><br>We do not know whether children are more susceptible to the effects of RDX than adults.<br><br>We do not know whether RDX causes birth defects in humans. |
| **Laboratory animals** | Exposure of animals to RDX during pregnancy has not caused birth defects in newborn animals. However, rats exposed to RDX during gestation gave birth to babies with smaller weight and length than rats not exposed to RDX.<br><br>In rats exposed to RDX during pregnancy, RDX was able to pass through the placenta and reached the fetus.<br><br>Young deer mice (21 days old) were more sensitive than older deer mice (50 days old) to the acute toxic effects of RDX. |
| **Breast milk** | There are no studies that looked for RDX in human breast milk. However, rats exposed to RDX during pregnancy had RDX in their milk, suggesting that the same can occur in humans. This means that women exposed to RDX who nurse their babies could transfer RDX to the babies in the milk. |

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

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| **Consumer products** | RDX is not found in consumer products. Therefore, families are not expected to have contact with RDX through the use of consumer products. |
| **Drinking water** | Families whose tap or well water may be contaminated with RDX may choose to drink or cook with bottled water or to install activated carbon water filters. |

## 1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO RDX?

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| **Detecting exposure** | RDX can be measured in blood and urine, but these are not routine tests that can be performed in a doctor's office.<br><br>We do not know whether the presence of RDX in the blood indicates that you were exposed briefly a few days before the blood was collected or that you are experiencing constant exposure. |
| **Measuring exposure** | The tests for RDX in blood and urine cannot be used to determine how much RDX entered your body.<br><br>The presence of RDX in your blood does not necessarily mean that you will suffer adverse health effects. The usual immediate health effects are seizures, muscle twitching, or vomiting from very high exposures. These would probably occur before you had the blood or urine test. |

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

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but cannot be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels. These are levels of a toxic substance in air, water, soil, or food that do not exceed a critical value. This critical value is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

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 provides it.

Some regulations and recommendations for RDX include the following:

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| **Levels in drinking water set by EPA** | The EPA has determined that exposure to RDX in drinking water at concentrations of 0.1 mg/L for one day or 0.1 mg/L for 10 days is not expected to cause any adverse effects in a child.<br><br>The EPA has determined that lifetime exposure to 0.002 mg/L RDX is not expected to cause any adverse effects. |
| **Levels in workplace air set by OSHA** | OSHA had previously set a legal limit of 1.5 $mg/m^3$ for RDX in March 1989; however, the standard was vacated in 1992.<br><br>NIOSH has set a 10-hour time-weighted average recommended exposure limit of 1.5 $mg/m^3$. |

## 1.10 WHERE CAN I GET MORE INFORMATION?

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

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.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may 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), by e-mail at cdcinfo@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology and Environmental Medicine
1600 Clifton Road NE
Mailstop F-62
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

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/

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# 2. RELEVANCE TO PUBLIC HEALTH

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

RDX is a military explosive produced by the nitrolysis of hexamine with nitric acid. It is a synthetic compound that does not occur naturally in the environment. Effluents and emissions from Army ammunition plants and many current and former military installations are responsible for the release of RDX into the environment. RDX can enter the air, water, and soil as a consequence of these releases. RDX is expected to exist as a particulate in the atmosphere. RDX has low water solubility and is subject to photolysis (half-life of 9–13 hours). RDX undergoes biodegradation in water and soil under anaerobic conditions to form several biodegradation products. RDX is mobile in soil and can leach into groundwater, and can be transported from soils or water to terrestrial and aquatic plants. RDX is not very lipid soluble, and therefore, has a low potential for bioaccumulation in aquatic species.

RDX has been identified in environmental samples, primarily near army munitions depots. Indoor air samples collected at ammunition plants were found to contain RDX in concentrations ranging from 0.032 to 60 $mg/m^3$. In water, RDX has been identified in a variety of groundwater samples from ammunition plants in the United States (<20–43,200 μg/L) and Germany (21–3,800 μg/L). Sediment samples from Army depots have been found to contain RDX in concentrations ranging from <0.1 to 3,574 mg/kg and in composts prepared from contaminated sediments (>2.9–896 mg/kg). Additionally, RDX was identified in plant species irrigated with or grown in contaminated water (<20–3,196 μg/L).

For the general population, including children, exposure to RDX is limited to areas around Army ammunition plants where it is manufactured, used in munitions, packed, loaded, or released through the demilitarization of antiquated munitions. The most likely route of exposure is ingestion of contaminated drinking water or agricultural crops irrigated with contaminated water. Exposure can also occur though dermal contact with soil containing RDX or by inhaling contaminated particulate matter produced during incineration of RDX-containing waste material. Children playing in contaminated water or soil may also be exposed via ingestion. Children can also be exposed if workers inadvertently bring home RDX adhered to shoes or clothing.

Occupational exposure to RDX can occur when workers handle RDX at Army ammunition plants. Under these conditions, exposure can occur as a result of release of dust into the workroom air, principally

during dumping of dried RDX powder, screening and blending, and clean-up of spilled material. Exposure to RDX can also occur through dermal contact during manufacture, handling, and clean-up of RDX. RDX was detected at a concentration of 0.052 mg/m$^3$ (0.47 ppm) in the particulate fraction of only one of eight indoor air samples taken from the incorporation area of Holston Army Ammunition Plant in Tennessee in 1986. Based on the observed concentration, the potential for exposure to RDX is considered to be negligible.

## 2.2 SUMMARY OF HEALTH EFFECTS

There is limited information on the toxicity of RDX in humans; the database consists of studies of workers exposed to RDX dust, soldiers using C-4 (a plasticized explosive containing 91% RDX) as a cooking fuel, and case reports of individuals ingesting RDX. Most of these studies involve acute exposure to RDX and provide limited exposure information. Neurologic dysfunction, primarily seizures and convulsions, was the most commonly reported effect. The seizures/convulsions typically occurred within several hours of exposure, and in some cases, convulsions were noted for several days after exposure. Other neurological symptoms that have been observed in humans include disorientation, lethargy, muscle twitching, and marked hyperirritability.

Studies in laboratory animals support neurological effects as a sensitive end point of RDX. Seizures, convulsions, and tremors have been reported in rats, deer mice, dogs, and monkeys orally exposed to RDX for acute, intermediate, or chronic durations. As with human exposure, the clonic-tonic convulsions and seizures are often observed shortly after exposure; however, a study in monkeys did not report seizures in some of the animals until after 34–57 doses of 10 mg/kg/day. In acute-exposure studies, the lowest adverse effect level for seizures and convulsions was 17 mg/kg/day, with no seizures at 12.5 mg/kg/day. In addition to these neurological effects, decreases in motor activity and impaired learning were observed in rats following administration of a single gavage dose of 12.5 mg/kg/day; however, no alterations in motor activity were observed in rats administered 10 mg/kg/day for 16 or 30 days. A lower adverse effect level (8 mg/kg/day) was reported in an intermediate-duration study, with a no-observed-adverse-effect level (NOAEL) of 4 mg/kg/day. At higher doses (≥40 mg/kg/day), hyperactivity, hyperirritability, hyperreactivity, and increased fighting have been observed in rats. Although the database is mostly comprised of studies in rats, intermediate-duration studies in monkeys and dogs do not suggest species differences in RDX-induced seizures/convulsions. In chronic-duration oral studies, seizures and convulsions were observed at 40 mg/kg/day; this dose was also associated with 88% lethality. No neurological effects were observed in rats chronically exposed to 8 or 10 mg/kg/day.

The animal data suggest that there may be other targets of RDX toxicity, including the hematological system and liver following oral exposure. Small, although significant, decreases in hemoglobin and erythrocyte levels were observed following intermediate-duration exposure, but this was not consistently found in other intermediate or chronic studies. Several studies found minor changes in serum chemistry parameters suggestive of a slight impairment of liver function. These alterations include decreases in alanine aminotransferase and in serum triglyceride and/or cholesterol levels and increases in serum cholesterol levels. A decrease in blood glucose levels observed in one study of rats may also be related to impaired liver function. Hepatomegaly and hepatocellular vacuolization have been reported in rats; however, most studies did not report histological alterations in the liver. An intermediate-duration study in monkeys reported an increase in vomiting following gavage administration of 10 mg/kg/day RDX; the occurrence of vomiting at 0.1 or 1 mg/kg/day was similar to controls.

There is limited information to suggest that RDX is a reproductive toxicant following oral exposure. An increased incidence of spermatic granuloma in the prostate was observed in rats following exposure to 40 mg/kg/day for 6 months; however, this effect was not observed at longer durations (1 or 2 years) in the same study. A nonsignificant increase in testicular degeneration was also observed in this study in rats exposed for 6 months to 40 mg/kg/day, but testicular effects were not observed in another study of rats exposed to 100 mg/kg/day for 13 weeks. Adverse developmental effects have been observed in rats, particularly at maternally toxic doses. Decreases in pup survival and increases in the occurrence of stillbirths were observed at 50 mg/kg/day; this dose also resulted in maternal deaths. A decrease in pup body weight and an increase in the incidence of renal cysts were observed in $F_2$ pups at 16 mg/kg/day in a two-generation study of rats and a decrease in fetal body weight and length were observed in the offspring of rats administered 20 mg/kg/day on gestation days 6–15. No adverse developmental (or maternal) effects were observed in rabbits.

The carcinogenic potential of RDX was evaluated in orally exposed rats and mice; no evidence of carcinogenicity was observed in two rat studies. In mice, an increase in the combined incidence of hepatocellular adenomas and carcinomas was observed in females only. However, a re-evaluation of these data using current diagnostic criteria resulted in a reclassification of some hepatocellular adenomas as foci of cytoplasmic alterations. As a result of the re-analysis, the combined incidence was significantly higher than concurrent controls at 35 mg/kg/day, but not at 100 mg/kg/day and the incidence in the 35 mg/kg/day group was within the range of historical control data. The investigators suggested that the study provided equivocal evidence of carcinogenicity. The International Agency for Research on Cancer

(IARC) and the Department of Health and Human Services (DHHS) have not classified the carcinogenicity of RDX. EPA classified RDX as a group C carcinogen, possibly carcinogenic to humans; however, this evaluation was done prior to the re-evaluation of mouse tumor data. EPA is currently re-evaluating RDX.

## 2.3 MINIMAL RISK LEVELS (MRLs)

ATSDR has made estimates of exposure levels posing minimal risk to humans (MRLs) for RDX. 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 adverse 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 levels posing minimal risks to humans (Barnes and Dourson 1988; EPA 1990b), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for adverse 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.

### *Inhalation MRLs*

ATSDR has not derived inhalation MRLs due to the limited data available on the toxicity of RDX following inhalation exposure. Several studies reported convulsions in humans acutely exposed to unspecified amounts of RDX (Hollander and Colbach 1969; Kaplan et al. 1965; Testud et al. 1996a). Nausea and vomiting have also been reported in humans (Hollander and Colbach 1969; Ketel and Hughes 1972); however, these individuals may have been exposed to RDX via inhalation and ingestion. Deaths due to bronchopneumonia, pneumonia, or pulmonary congestion were observed in rabbits and guinea pigs exposed to an unspecified concentration of RDX (Sunderman 1944). The lack of dose-response data for the human and animal studies precludes derivation of inhalation MRLs.

***Oral MRLs***

***Acute-Duration***

- ATSDR has derived an MRL of 0.2 mg/kg/day for acute-duration oral exposure (14 days or less) to RDX. This MRL is based on a NOAEL of 8.5 mg/kg/day and LOAEL of 17 mg/kg/day for convulsions/seizures observed in rats administered RDX via gavage 7 days/week for 14 days (U.S. Army 2006). A PBPK model was used to predict peak brain concentrations in the rat and to estimate human equivalent doses (HEDs). The $NOAEL_{HED}$ of 6.45 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

The acute toxicity database consists of several human exposure studies reporting convulsions and seizures following oral exposure to RDX (Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986). Although some studies provide exposure estimates, these values are not considered reliable. Animal studies have also identified convulsions and seizures as the most sensitive effect following acute-duration oral exposure (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1980b, 1986d, 2006). The lowest adverse effect level for convulsions/seizures was 17 mg/kg/day in rats administered RDX via gavage for 14 days (U.S. Army 2006); an increase in the incidence of mortality was also observed at this dose level (U.S. Army 2006). Several other studies have identified similar lowest-observed-adverse-effect levels (LOAELs); convulsions were observed in rat dams administered via gavage 20 mg/kg/day on gestation days 6–15 or 19 (U.S. Army 1980b, 1986d) and seizures were observed in rats administered a single gavage dose of 25 mg/kg (Burdette et al. 1988). The dose-response curve for seizures/convulsions appears to be fairly steep, with no effects at 8.5 (U.S. Army 2006) or 12.5 (U.S. Army 1985b) mg/kg/day and seizures at 17 mg/kg/day (U.S. Army 2006). Additionally, decreases in motor activity and learning were observed at a lower dose (12.5 mg/kg/day) in rats receiving a single gavage dose (U.S. Army 1985b).

There are limited data on the non-neurological toxicity of RDX following acute-duration exposure. U.S. Army (2006) monitored body weight, hematological parameters, clinical chemistry parameters, and organ weight in male and female rats administered gavage doses of 2–17 mg/kg/day for 14 days. No biologically relevant alterations in these systemic toxicity end points were observed. Decreases in fetal weight and length were observed in the offspring of rats administered 20 mg/kg/day on gestation days 6–15 (U.S. Army 1986d); however, this exposure was associated with maternal convulsions/seizures and death.

## 2. RELEVANCE TO PUBLIC HEALTH

Based on the available data, impaired neurological function was identified as the critical effect for derivation of an acute-duration oral MRL. Although the acute database lacks studies adequately assessing systemic toxicity, intermediate-duration studies have found no systemic effects at doses lower than those affecting the nervous system. The lowest adverse effect level for neurological effects is 12.5 mg/kg/day for decreases in motor activity and learning in rats following a single gavage dose (U.S. Army 1985b); this study did not identify a NOAEL. In a repeated exposure study by this group (U.S. Army 1985b), no significant alterations in motor activity were observed in rats following 15 or 30 days of exposure to doses as high as 10 mg/kg/day. At a slightly higher dose (17 mg/kg/day), convulsions and tremors were observed in rats administered RDX for 14 days (U.S. Army 2006); no neurological effects were observed at 8.5 mg/kg/day. The U.S. Army (2006) study was selected as the principal study because it identified a NOAEL and involved repeated exposure, and it is likely that an MRL based on this study would be protective for the neurobehavioural effects observed at 12.5 mg/kg/day in the U.S. Army (1985b) study. In the U.S. Army (2006) study, groups of 6 male and 6 female Sprague-Dawley rats were administered via gavage 0, 2.125, 4.25, 8.5, 17.00, 25.50, 34.00, or 42.5 mg/kg/day as a suspension of RDX/1% methylcellulose/0.2% Tween 80 in distilled water 7 days/week for 14 days. Rats were monitored daily for toxic signs and morbidity. Body weights and feed consumption were measured on days 0, 1, 3, 7, and 14. Additional parameters used to assess toxicity included clinical chemistry and hematology values, organ weights, and gross necropsies. A significant increase in early deaths was observed at ≥25.5 mg/kg/day. Tremors and convulsions were observed in rats exposed to ≥17 mg/kg/day. In the males exposed to ≥17 mg/kg/day, blood stains around the mouth and nose and low arousal were also observed. Increased arousal, blood around the mouth and nose, barbering, and lacrimation were observed in females exposed to ≥17 mg/kg/day. No signs of neurological alterations were observed in rats exposed to ≤8.5 mg/kg/day. Significant decreases in body weight were observed in male rats exposed to ≥17 mg/kg/day on days 1 and 7, but there were no significant alterations in male body weight at termination. In female rats, significant decreases in body weight gain were observed at ≥34 mg/kg/day on day 1 and in the 8.5 mg/kg/day group on day 14; however, the magnitude of the decreased body weight was <10% and no significant alterations were observed at higher dose levels. Significant decreases in food consumption were also observed during the first 7 days of exposure in males and females exposed to ≥8.5 mg/kg/day. Significant decreases in absolute liver weights and liver-to-brain weights and increases in blood cholesterol levels were observed in females exposed to 8.5 mg/kg/day; these effects were not observed at higher dose levels or in males. Due to the lack of dose-response relationships for the alterations in liver weight and blood cholesterol levels, these changes observed in the 8.5 mg/kg/day

female group were not considered biologically relevant. No significant alterations in hematological parameters or other clinical chemistry parameters or organ weights were observed.

The acute-duration oral MRL was derived using the NOAEL/LOAEL approach; the lack of incidence data for the neurological effects precluded using a benchmark dose approach. The MRL is based on the NOAEL of 8.5 mg/kg/day and a LOAEL of 17 mg/kg/day identified in the U.S. Army (2006) study. The available mode of action data suggest that the induction of seizures and/or convulsions is likely associated with the binding of RDX to GABA receptors in the brain and the onset of seizures is directly related to the levels of RDX in the brain (Gust et al. 2009; Williams et al. 2011). A physiologically based pharmacokinetic (PBPK) model (Sweeney et al. 2012) was used to predict peak brain RDX concentration and mean brain RDX concentration for each administered dose in the U.S. Army (2006) study. Based on a comparison of predicted brain RDX concentrations to the NOAEL and LOAEL values for seizures/convulsions observed in intermediate and chronic studies, ATSDR determined that peak brain RDX concentration was a more appropriate internal dose metric for derivation of the MRL than mean brain RDX concentration. To determine the point of departure for the MRL, the PBPK model was used to predict HEDs from peak brain concentration data. Detailed discussions of the PBPK model and support for using peak brain concentration as the internal dose metric are presented in Appendix A. The PBPK model predicted a peak brain concentration of 6.19 mg/L in rats administered 8.5 mg/kg/day 7 days/week for 14 days and a HED of 6.455 mg/kg/day. The MRL of 0.2 mg/kg/day was calculated by dividing the $NOAEL_{HED}$ of 6.45 mg/kg/day for neurological effects (U.S. Army 2006) by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

***Intermediate-Duration***

- ATSDR has derived an MRL of 0.1 mg/kg/day for intermediate-duration oral exposure (15–364 days) to RDX. The MRL is based on a 90-day study which identified a NOAEL of 4 mg/kg/day and LOAEL of 8 mg/kg/day for seizures/convulsions in rats receiving gavage doses of RDX 7 days/week (U.S. Army 2006). ATSDR derived the MRL using benchmark dose modeling of seizure/convulsion incidence data and PBPK modeling to predict peak brain concentrations in the rat and to estimate HEDs. The $BMCL_{HED}$ of 4.13 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

No human studies have examined the toxicity of RDX following intermediate-duration exposure. Data from laboratory animal studies suggest that the nervous system is the most sensitive target of RDX toxicity. Convulsions, seizures, and/or tremors have been observed in rats at doses of ≥8 mg/kg/day (U.S.

Army 1983a, 2006; von Oettingen et al. 1949), monkeys at 10 mg/kg/day (U.S. Navy 1974b), and dogs at 50 mg/kg/day (von Oettingen et al. 1949). In addition, hyperactivity was noted in rats exposed to 100 mg/kg/day (Levine et al. 1981, 1990). The results of the U.S. Army (2006) study suggest that there is a steep dose-response curve for seizure induction. The occurrences of seizures were 0% at 4 mg/kg/day, 20–30% at 8 mg/kg/day, 45–50% at 10 mg/kg/day, and 80–90% at 12 or 15 mg/kg/day. An increase in mortality was often reported at the lowest doses associated with seizures. Less serious adverse health effects have been observed at similar or higher dose levels. Several studies have found changes in serum chemistry parameters suggestive of impaired liver function, although histological alterations were not generally found in the liver. Decreases in serum cholesterol and/or triglycerides were observed at ≥8 mg/kg/day (U.S. Army 1983a, 2006; Levine et al. 1981) and decreases in serum alanine aminotransferase activity levels were observed at 28 mg/kg/day (U.S. Army 1980b). The magnitude of these alterations was small and not likely to be biologically significant. Small, although significant, decreases in erythrocyte and hemoglobin levels were also observed in rats exposed to 40 mg/kg/day (U.S. Army 1983a) and mice exposed to 160 mg/kg/day (U.S. Army 1980b), but this finding has not been consistently found in intermediate-duration studies (U.S. Army 1980b, 2006; von Oettingen et al. 1949). Emesis was observed in monkeys administered via gavage 10 mg/kg/day for 90 days (U.S. Navy 1974b); the incidence in monkeys administered 1 mg/kg/day was not considered to be different from the controls. There is limited evidence that RDX is a reproductive toxicant. An increased incidence of spermatic granuloma was observed in the prostate of rats exposed to 40 mg/kg/day for 6 months (U.S. Army 1983a). In a two-generation study in rats, decreases in $F_2$ pup body weight and increases in the incidence of renal cysts were observed at 16 mg/kg/day and increases in the number of stillbirths and decreases in pup survival were observed in the $F_1$ generation exposed to 50 mg/kg/day (U.S. Army 1980b).

Impaired neurological function was identified as the critical effect for derivation of an intermediate-duration oral MRL. The lowest adverse effect level for this end point is 8 mg/kg/day with a NOAEL of 4 mg/kg/day (U.S. Army 2006). A slightly higher LOAEL of 10 mg/kg/day was identified in monkeys (U.S. Navy 1974b); a NOAEL was not identified in this study. The rat study (U.S. Army 2006) was selected as the principal study. In this study, groups of 10 male and 10 female F344 rats were administered via gavage 0, 4, 8, 10, 12, or 15 mg/kg/day as a suspension of RDX/1% methylcellulose/ 0.2% Tween 80 in distilled water 7 days/week for 90 days. Rats were monitored weekly for toxic signs and functional observational battery (FOB) observations (home-cage, hand held, and open arena observations), and body weights and feed consumption were measured weekly. Additional parameters used to assess toxicity included neurobehavioral tests after week 11 (motor activity, grip strength, and sensory reactivity to different types of stimuli), ophthalmic examination, urinalysis, clinical chemistry,

hematology, coagulation, organ weights, gross necropsies, and histopathological examination of major tissues and organs from rats exposed to 0 or 15 mg/kg/day. Significant increases in mortality rates were observed at ≥10 mg/kg/day. Convulsions were observed in most animals dying early. Transient clinical signs included changes in arousal, inflammation of eyelash follicles, increased salivation, blood stains around mouth and nose, rough haircoat, tremors, and convulsions; the incidence and severity increased with dose. The incidences of convulsions were 0/20, 0/20, 3/20, 6/20, 13/20, and 12/20 in rats exposed to 0, 4, 8, 10, 12, and 15 mg/kg/day, respectively. Although the incidence of convulsions was not statistically significant at 8 mg/kg/day, the increased incidence of seizures was considered biologically significant and the 8 mg/kg/day dose level was considered a LOAEL. The tremors/convulsions were observed within the first week of exposure in the 12 or 15 mg/kg/day groups and persisted throughout the study. No significant RDX-related alterations in foot splay, front limb grip strength, or response to stimuli were found. Hematological tests showed significant increases in erythrocyte mean cell volume at 8 (males only), 10, and 12 mg/kg/day and significant decrease in serum cholesterol in males exposed to ≥8 mg/kg/day. No significant increases in the incidence of histopathological alterations were observed.

The intermediate-duration oral MRL was derived using benchmark dose modeling and PBPK modeling. As discussed for the acute-duration oral MRL, a PBPK model (Sweeney et al. 2012) was used to predict peak and mean brain RDX concentrations. Comparisons with empirical seizure data following intermediate- or chronic-duration exposure with predicted brain RDX levels provided support for using peak brain concentration as the internal dose metric for the MRL derivation. The incidence data for convulsions in rats were fit to several dichotomous models using a benchmark response (BMR) of 10% and the internal dose metric of peak brain concentration. Detailed discussion of the benchmark dose modeling, the PBPK model, and support for the selection of the internal dose metric are presented in Appendix A. The log-probit model provided the best fit to the data and was used to estimate a 95% lower confidence limit on the benchmark dose ($BMDL_{10}$) of 3.9624 mg/L. Using PBPK modeling, a HED of 4.1308 mg/kg/day was predicted from the $BMDL_{10}$. The MRL of 0.1 mg/kg/day was calculated by dividing the $BMDL_{HED}$ of 4.1308 mg/kg/day for neurological effects by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

***Chronic-Duration***

- ATSDR has derived an MRL of 0.1 mg/kg/day for chronic-duration oral exposure (≥365 days) to RDX. This MRL is based on a NOAEL of 8 mg/kg/day and LOAEL of 40 mg/kg/day for convulsions/seizures observed in rats exposed to RDX in the diet for 2 years (U.S. Army 1983a). A PBPK model was used to predict peak brain concentrations in the rat and to estimate HEDs.

The $NOAEL_{HED}$ of 4.223 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability).

The chronic oral toxicity of RDX has been evaluated in two rat studies (U.S. Army 1983a; U.S. Navy 1976) and a mouse study (U.S. Army 1984c). A number of adverse health effects have been observed in rats exposed to 40 mg/kg/day including tremors, convulsions, and hyperresponsiveness; decreased hematocrit, hemoglobin, and erythrocyte levels; hepatomegaly and decreased serum cholesterol and triglycerides; renal papillary necrosis and increased blood urea nitrogen levels; testicular degeneration; and cataracts (females only) (U.S. Army 1983a); no adverse effects were observed in rats exposed to 8 mg/kg/day. The 40 mg/kg/day dose was also associated with an 88% mortality rate. In addition to these effects, significant increases in the incidence of suppurative inflammation were observed in the prostate of rats exposed to ≥1.5 mg/kg/day (U.S. Army 1983a). U.S. Army (2006) noted that inflammation of the prostate gland is a common condition in older rodents and is generally not due to toxicity; additionally, the prostate effects in the U.S. Army (1983a) study were predominantly found in rats dying early.

In the U.S. Navy (1976) rat study, no adverse effects were observed at doses as high as 10 mg/kg/day. This study did not include a histological examination of the prostate and the animals were monitored weekly for overt signs of toxicity. In mice, increases in serum cholesterol levels were observed in females exposed to 35 mg/kg/day and increased relative kidney weights and cytoplasmic vacuolization in the kidney were observed at 100 mg/kg/day (U.S. Army 1984c). The toxicological significance of the increased serum cholesterol level in the absence of other indications of hepatic damage is not known.

The lowest LOAEL identified in chronic-duration studies is 1.5 mg/kg/day for prostate inflammation; the NOAEL for this effect is 0.3 mg/kg/day. However, U.S. Army (1983a) suggested that this effect is likely secondary to a bacterial infection in older rats dying early; thus, it was not considered an appropriate basis of a chronic-duration MRL. The effects observed in rats (including convulsions/tremors, hematological alterations, impaired hepatic function, and renal lesions) exposed to 40 mg/kg/day (NOAEL of 8 mg/kg/day) were considered as the basis of a chronic-duration MRL. Based on a comparison of the effects observed in rats exposed to 40 mg/kg/day and those observed in mice exposed to 35 mg/kg/day, rats appear to be more sensitive to the toxicity of RDX than mice; thus, the mouse study was not considered for MRL derivation.

In the U.S. Army (1983a) study, groups of male and female Fischer 344 rats (75/sex/group) were exposed to 0, 0.3, 1.5, 8.0, or 40.0 mg/kg/day RDX in the diet for 2 years. The following parameters were used to assess toxicity: daily observations, ophthalmic examinations, hematology, clinical chemistry, organ weights, and complete histopathology of major tissues and organs of rats in the 0 or 40.0 mg/kg/day groups, and histopathological examination of the brain, gonads, heart, liver, kidneys, spleen, and spinal cord of rats in the 0.3, 1.5, and 8.0 mg/kg/day groups. Actual RDX doses were within 3% of the intended dose. Deaths were observed at 40 mg/kg/day; 88% of males and 41% of females died by week 88. The mean survival time for the 40 mg/kg/day males was 14.6 months compared with 22.3 months for the control males. A 20.6 month survival time was seen for the 40 mg/kg/day females vs. 22.0 months for the control females at 40 mg/kg/day. A significant decrease in survival time was also observed in the males exposed to 1.5 mg/kg/day (21.0 months); however, no alterations in survival time was observed in the females exposed to 1.5 mg/kg/day (22.2 months) or in the males (22.2 months) or females (22.4 months) exposed to 8 mg/kg/day. Additionally, there were no significant differences in mortality incidence in the 1.5 or 8 mg/kg/day groups, as compared to controls. Statistically decreased body weight gain was observed in males (20–30%) and females (10–15%) exposed to 40.0 mg/kg/day; statistically significant decreases in body weight gain were also observed at 8.0 mg/kg/day, but the body weight was within 10% of controls. Tremors and convulsions were observed prior to death at 40 mg/kg/day; the animals were hyperactive to approach and had increased fighting. No adverse clinical signs were noted for the lower dose groups. Significant decreases in hemoglobin and erythrocyte counts were observed in the 40 mg/kg/day group beginning at week 26; the study investigators noted that the anemic state was considered slight and there was no evidence of physiologic compensatory responses. Thrombocytosis was observed in rats exposed to 40 mg/kg/day and elevated platelet counts were observed in 8 mg/kg/day males during weeks 13 and 26. Significant decreases in blood glucose, total cholesterol, and triglyceride levels were observed in the 40 mg/kg/day group starting at week 13. Significant decreases in serum alanine aminotransferase levels were observed in males exposed to 8 or 40 mg/kg/day at weeks 26 and 52 and in females at 40 mg/kg/day at week 26. Other clinical chemistry alterations included decreases in globulin and albumin levels at weeks 52 and 78 and increases in serum potassium levels at weeks 26, 52, and 78. A significant increase in the incidence of cataracts was observed in females in the 40 mg/kg/day group during weeks 78 and 104. Histological alterations observed after 2 years of exposure included suppurative inflammation of the prostate in the 1.5, 8, and 40 mg/kg/day groups; renal medullary papillar necrosis, renal pyelitis, and urinary bladder luminal distension and cystitis in males exposed to 40 mg/kg/day; splenic extramedullary hematopoiesis in female rats exposed to 40 mg/kg/day; and hemosiderin-like pigment in males exposed to 1.5, 8, or 40 mg/kg/day. In the absence of altered hematological parameters or other effects on the spleen, the increased pigment levels observed at 1.5 or 8 mg/kg/day were not considered adverse.

The U.S. Army (1983a) study identified a NOAEL of 8 mg/kg/day and a LOAEL of 40 mg/kg/day for tremors and convulsions in rats exposed to RDX in the diet for 2 years. ATSDR derived a chronic-duration oral MRL using the NOAEL/LOAEL approach; benchmark dose modeling could not be utilized because the investigators did not report incidence data for neurological signs. As discussed for the acute-duration oral MRL, a PBPK model (Sweeney et al. 2012) was used to predict peak and mean brain RDX concentrations. Comparisons with empirical seizure data following intermediate- or chronic-duration exposure with predicted brain RDX levels provided support for using peak brain concentration as the internal dose metric for the MRL derivation. To determine the point of departure for the MRL, the PBPK model was also used to predict the HED for a given rat peak brain RDX concentration; detailed discussions of the PBPK model and support for selecting peak brain RDX concentration as the internal dose metric are presented in Appendix A. The NOAEL from the U.S. Army (1983a) study corresponds to peak brain concentrations of 4.051 mg/L and a HED of 4.223 mg/kg/day. The MRL of 0.1 mg/kg/day was calculated by dividing the $NOAEL_{HED}$ of 4.223 mg/kg/day by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability).

# 3. HEALTH EFFECTS

## 3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of RDX. 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

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

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of RDX are indicated in Table 3-1 and Figure 3-1. Because cancer effects could occur at lower exposure levels, Figure 3-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 ($10^{-4}$ to $10^{-7}$), as developed by EPA.

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

### 3.2.1 Inhalation Exposure

#### 3.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to RDX. Death attributed to impairment of the respiratory system was observed in rabbits and guinea pigs exposed to an unspecified concentration of RDX (Sunderman 1944).

#### 3.2.1.2 Systemic Effects

Four studies were located regarding systemic effects in humans after inhalation exposure to RDX alone. The available studies have reported adverse gastrointestinal, hematological, hepatic, and renal effects in workers exposed to C-4 (an explosive composed of 91% RDX) or RDX dusts via inhalation. Since the exposure concentration and/or duration were not described for these studies, they are not presented in tables or figures. No studies were located regarding respiratory, cardiovascular, musculoskeletal, dermal, ocular, or other systemic effects in humans after inhalation exposure to RDX. Case reports are available regarding systemic effects in workers exposed to unknown levels of RDX via the inhalation or oral routes

(Ketel and Hughes 1972). These studies are also discussed in Section 3.2.2.2. Only one study is available regarding systemic effects in animals after inhalation exposure to RDX (Sunderman 1944). This study is limited by insufficient numbers of animals tested, no controls, and no data on exposure levels. No studies were located regarding gastrointestinal, hepatic, or dermal effects in animals.

**Respiratory Effects.** Three of 6 rabbits died from bronchopneumonia; death of 7 of 18 guinea pigs was attributed to pneumonia and pulmonary congestion (Sunderman 1944).

**Cardiovascular Effects.** Histopathology revealed the absence of striations in the cardiac muscle of guinea pigs exposed to unspecified levels of RDX for 4–67 days (Sunderman 1944).

**Gastrointestinal Effects.** Soldiers who were exposed to an unspecified amount of C-4 (91% RDX) as a cooking fuel for an unknown duration experienced nausea and vomiting (Hollander and Colbach 1969; Ketel and Hughes 1972); the soldiers were exposed to RDX via the inhalation and/or oral routes.

**Hematological Effects.** Two studies of workers exposed to RDX dusts are available, but neither revealed any adverse hematological effects. In one study, workers who were presumably exposed acutely to unknown levels of RDX dusts had normal blood counts (Kaplan et al. 1965). In the other study, workers exposed to an average of 0.28 $mg/m^3$ of RDX dusts in the workplace, presumably for a chronic period, showed no hematological changes compared to controls (Hathaway and Buck 1977). Transient elevation of the white blood count was frequently observed in individuals exposed to C-4 (91% RDX). Normal red blood count, leukocytes, and hemoglobin were reported in rats following intermediate exposure to RDX. However, in the same study, hemoglobin counts were decreased in guinea pigs (Sunderman 1944).

**Hepatic Effects.** No liver toxicity was revealed by blood or urine analyses of workers exposed to RDX in the air; the duration of exposure was not reported (Hathaway and Buck 1977).

**Renal Effects.** Blood and urine analyses of workers exposed to RDX in the air for acute (Kaplan et al. 1965) or chronic durations (Hathaway and Buck 1977) did not reveal any kidney toxicity. Although no renal toxicity was observed after exposure to RDX dust, there were some manifestations of renal damage after possible inhalation exposure to C-4 (91% RDX): transient oliguria and proteinuria in two patients and acute renal failure in one case (Ketel and Hughes 1972).

There was no kidney pathology in rats or guinea pigs exposed to RDX, but degeneration of the kidneys was found in rabbits exposed to unspecified levels of RDX for an intermediate period (Sunderman 1944). This study is limited in that no controls were used, and details of the study were not specified.

#### 3.2.1.3 Immunological and Lymphoreticular Effects

Workers at an Army ammunition plant who were exposed to an average of 0.28 mg/m$^3$ of RDX dusts for an unknown period of time showed no significant differences in a test for antinuclear antibodies as compared to nonexposed workers. The results of this test provide no evidence of autoimmune disease (Hathaway and Buck 1977). No other immunological function tests were performed.

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

#### 3.2.1.4 Neurological Effects

Convulsions and unconsciousness, accompanied by headache, dizziness, and vomiting, were noted in 5 out of 26 workers who were exposed to unknown levels of RDX dust in the air (Kaplan et al. 1965). Similar findings, such as convulsions, muscle twitching, and confusion, have been reported in five case studies of men exposed to C-4 fumes (91% RDX) when it was used as a cooking fuel (Hollander and Colbach 1969), and in a worker hand-sieving RDX (Testud et al. 1996a). The workers recovered a few days after they were removed from the source of exposure. Testud et al. (1996a) noted that CT scan and MRI (performed 1 week after exposure) were normal and electroencephalogram only showed signs of the administered anticonvulsant therapy; in the other studies, tests of neurological function were not performed. In a study of workers at an RDX facility, no increases in the occurrence of subjective symptoms were reported (Ma and Li 1993). Significant differences in performance on tests of memory retention and block design were found in workers exposed to 0.407 or 0.672 mg/m$^3$, as compared to controls; however, no differences were found between the two exposed groups. No significant alterations in performance on tests of reaction time were noted.

No studies were located regarding neurological effects in animals after inhalation exposure to RDX.

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

#### 3.2.1.5 Reproductive Effects

#### 3.2.1.6 Developmental Effects

#### 3.2.1.7 Cancer

### 3.2.2 Oral Exposure

#### 3.2.2.1 Death

No studies were located regarding death in humans after oral exposure to RDX.

Deaths were reported in animals following acute, intermediate, and chronic exposures to RDX. Three out of 12 rats died during induced seizures following acute exposure to 50 mg/kg RDX, which was administered by gavage (Burdette et al. 1988). $LD_{50}$ values for single gavage doses were 71–118 mg/kg in rats (U.S. Army 1978b, 1980b), 86–97 mg/kg in mice (U.S. Army 1978b, 1980b), and 136–319 mg/kg in deer mice (Smith 2007). Apparent age-related differences in $LD_{50}$ values were found in deer mice; the $LD_{50}$ values were 136, 319, and 158 mg/kg in 21-, 50-, and 200-day-old mice (Smith et al. 2007). Miniature swine died (2/10) following single gavage doses of 100 mg/kg (Schneider et al. 1977). Rat dams that were fed 20 mg/kg/day of RDX during gestation had mortality rates of 24% (U.S. Army 1980b, 1986d).

In 90-day feeding studies, levels as low as 25 mg/kg/day (von Oettingen et al. 1949) and 100 mg/kg/day, produced deaths in rats (Levine et al. 1990), and levels of 320 mg/kg/day produced deaths in mice (U.S. Army 1980b). Increased mortality (25%) was observed in rats administered via gavage 10 mg/kg/day (U.S. Army 2006); however, no deaths were observed in dogs (U.S. Navy 1974a) or monkeys (U.S. Navy 1974b) also administered 10 mg/kg/day. In chronic-duration studies, an excessive number of deaths was observed in rats exposed to 40 mg/kg/day for 1–2 years compared to controls (U.S. Army 1983a). However, an excessive number of deaths was not observed in rats administered 10 mg/kg/day of RDX (U.S. Navy 1976). The $LD_{50}$ values and all reliable LOAEL values for death are recorded in Table 3-1 and plotted in Figure 3-1.

Table 3-1 Levels of Significant Exposure to RDX - Oral

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | | |
| **Death** | | | | | | | | |
| 1 | Rat (Long- Evans) | once (GW) | | | | 50 M (3/12 died during seizures) | Burdette et al. 1988 | |
| 2 | Rat (Sprague-Dawley) | once (GW) | | | | 50 (2/10 died) | Schneider et al. 1977 | |
| 3 | Rat (Sprague-Dawley) | once (GO) | | | | 71 M (LD50 )<br>75 F (9/10 rats died) | U.S. Army 1978b | |
| 4 | Rat (Fischer 344) | Gd 6-19 (GW) | | | | 20 F (6/25 died) | U.S. Army 1980b | |
| 5 | Rat (Fischer 344) | once (G) | | | | 119 (LD50) | U.S. Army 1980b | |
| 6 | Rat (Sprague-Dawley) | Gd 6-15 (GW) | | | | 20 F (31% died) | U.S. Army 1986d | |
| 7 | Rat (Sprague-Dawley) | 7 d/wk 14 d (GW) | | | | 25.5 (75% mortality) | U.S. Army 2006 | |
| 8 | Rat (NS) | once (GW) | | | | 100 (40% mortality) | von Oettingen et al. 1949 | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **9** | Mouse (Swiss-Webster) | once (GO) | | | | 86 M (LD50)<br>75 F (5/10 mice died) | U.S. Army 1978b | |
| **10** | Mouse (B6C3F1) | once (G) | | | | 97 M (LD50)<br>59 F (LD50) | U.S. Army 1980b | |
| **11** | Pig (NS) | once (GW) | | | | 100 F (2/10 died) | Schneider et al. 1977 | |
| **Systemic** | | | | | | | | |
| **12** | Rat (Fischer 344) | Gd 6-19 (GW) | Bd Wt | | 20 F (12% decrease in maternal body weight) | | U.S. Army 1980b | |
| **13** | Rat (Sprague-Dawley) | 7 d/wk 14 d (GW) | Hemato | 17 | | | U.S. Army 2006 | |
| **14** | Mouse (Peromyscus leucopus) | daily 14 days (F) | Hepatic | 68 F | | | EPA 1999 | |
| | | | Renal | 68 F | | | | |
| | | | Bd Wt | 68 F | | | | |
| **Neurological** | | | | | | | | |
| **15** | Human | once | | | | 357 M (seizures) | Stone et al. 1969 | |
| **16** | Rat (Long- Evans) | once (GW) | | 12.5 M | | 25 M (seizures) | Burdette et al. 1988 | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| 17 | Rat (Sprague-Dawley) | once (GO) | | | | 87 F (convulsions in 2/2 rats) | Meyer et al. 2005 | |
| 18 | Rat (Sprague-Dawley) | once (GW) | | | | 50 (convulsions) | Schneider et al. 1977 | |
| 19 | Rat (Fischer 344) | Gd 6-19 (GW) | | 2 F | | 20 F (convulsions and hyperactivity in dams) | U.S. Army 1980b | |
| 20 | Rat (Sprague-Dawley) | once (GW) | | | 12.5 (decreases in motor activity, taste aversion, learning, and auditory startle response amplitude) | | U.S. Army 1985b | |
| 21 | Rat (Sprague-Dawley) | Gd 6-15 (GW) | | 6 F | | 20 F (convulsions, prostration in dams) | U.S. Army 1986d | |
| 22 | Rat (Sprague-Dawley) | 7 d/wk 14 d (GW) | | 8.5[b] | | 17 (tremors and convulsions) | U.S. Army 2006 | |
| 23 | Pig (NS) | once (GW) | | | | 100 F (convulsions) | Schneider et al. 1977 | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **Developmental** | | | | | | | | |
| **24** | Rat (Fischer 344) | Gd 6-19 (GW) | | 2 F | | | U.S. Army 1980b | |
| **25** | Rat (Sprague-Dawley) | Gd 6-15 (GW) | | 6 F | 20 F (9% decrease in fetal weight and 5% decrease in fetal length) | | U.S. Army 1986d | |
| **INTERMEDIATE EXPOSURE** | | | | | | | | |
| **Death** | | | | | | | | |
| **26** | Rat (Fischer 344) | 13 wk (F) | | | | 100 (65% mortality) | Levine et al. 1981 | |
| **27** | Rat (Fischer 344) | 13 wk (F) | | | | 100 (13/20 died) | Levine et al. 1990 | |
| **28** | Rat (Fischer- 344) | 7 d/wk 90 d (GW) | | | | 10 (25% mortality) | U.S. Army 2006 | |
| **29** | Rat (NS) | 90 d (F) | | | | 25 (8/20 died) | von Oettingen et al. 1949 | |
| **30** | Rat (NS) | 10 wk (F) | | | | 50 (60% mortality) | von Oettingen et al. 1949 | |
| **31** | Mouse (B6C3F1) | 90 d (F) | | | | 320 M (4/10 died) | U.S. Army 1980b | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **Systemic** | | | | | | | | |
| **32** | Monkey (Cynomolgus) | 90 d 7 d/wk (GW) | Resp | 10 | | | U.S. Navy 1974b | |
| | | | Cardio | 10 | | | | |
| | | | Gastro | 1 | 10 (vomiting in 5/6 animals) | | | |
| | | | Hemato | 1 | 10 (necrotic and degenerate megakaryocytes in bone marrow) | | | |
| | | | Hepatic | 10 | | | | |
| | | | Renal | 10 | | | | |
| | | | Endocr | 10 | | | | |
| | | | Ocular | 10 | | | | |
| **33** | Rat (Fischer 344) | 13 wk (F) | Resp | 100 | | | Levine et al. 1981 | |
| | | | Cardio | 100 | | | | |
| | | | Gastro | 100 | | | | |
| | | | Hemato | | 10 F (increased leukocyte counts) | | | |
| | | | Hepatic | 10 | 30 (10-14% decrease in serum triglycerides) | | | |
| | | | Renal | 100 | | | | |
| | | | Bd Wt | 30 | 100 M (17% weight loss) | | | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **34** | Rat (Fischer 344) | 3-13 wk (F) | Resp | 100 | | | Levine et al. 1990 | |
| | | | Cardio | 100 | | | | |
| | | | Gastro | 100 | | | | |
| | | | Hemato | 100 | | | | |
| | | | Hepatic | | 30 (decr serum triglyceride levels) | | | |
| | | | Renal | 100 | | | | |
| | | | Bd Wt | | 30 M (13% decrease in body weight gain) | 100 M (29% decr body weight gain) | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **35** | Rat (Fischer 344) | 90 d (F) | Resp | 40 | | | U.S. Army 1980b | |
| | | | Cardio | 28 F | 40 F (decreased absolute heart weight, myocardial degeneration) | | | |
| | | | Gastro | 40 | | | | |
| | | | Hemato | 40 | | | | |
| | | | Musc/skel | 40 | | | | |
| | | | Hepatic | 40 | | | | |
| | | | Renal | 40 | | | | |
| | | | Endocr | 40 | | | | |
| | | | Dermal | 40 | | | | |
| | | | Ocular | 40 | | | | |
| | | | Bd Wt | 40 | | | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|---|
| **36** | Rat (Fischer 344) | 6 mo (F) | Resp | 40 | | | | U.S. Army 1983a | |
| | | | Cardio | 40 | | | | | |
| | | | Gastro | 40 | | | | | |
| | | | Hemato | 8 | 40 | (decreased hemoglobin and erythrocyte levels) | | | |
| | | | Musc/skel | 40 | | | | | |
| | | | Hepatic | 8 | 40 | (decreased serum triglyceride and cholesterol levels) | | | |
| | | | Renal | 40 | | | | | |
| | | | Endocr | 40 | | | | | |
| | | | Dermal | 40 | | | | | |
| | | | Ocular | 40 | | | | | |
| | | | Bd Wt | 8 | 40 | (17% decrease in body weight gain) | | | |
| | | | Metab | 8 | 40 | (decreased blood glucose levels) | | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **37** | Rat (Fischer- 344) | 7 d/wk 90 d (GW) | Resp | 15 | | | U.S. Army 2006 | |
| | | | Cardio | 15 | | | | |
| | | | Gastro | 15 | | | | |
| | | | Hemato | 15 | | | | |
| | | | Hepatic | 4 F | 8 M (decreased serum cholesterol levels) | | | |
| | | | Renal | 15 | | | | |
| | | | Ocular | 15 | | | | |
| | | | Bd Wt | 15 | | | | |
| **38** | Rat (NS) | 90 d (F) | Bd Wt | 15 | | 25 (weight loss) | von Oettingen et al. 1949 | |
| **39** | Rat (NS) | 10 wk (F) | Bd Wt | 15 | | 50 (weight loss) | von Oettingen et al. 1949 | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **40** | Mouse (B6C3F1) | 90 d (F) | Resp | 320 | | | U.S. Army 1980b | |
| | | | Cardio | 160 M | 320 M (slight myocardial degeneration) | | | |
| | | | Gastro | 320 | | | | |
| | | | Hemato | 80 M | 160 M (12% decrease in erythrocyte count and 7% decrease in hemoglobin concentration) | | | |
| | | | Hepatic | 160 M | 320 M (hepatocellular vacuolization) | | | |
| | | | Renal | 160 M | 320 M (mild tubular nephrosis) | | | |
| | | | Endocr | 160 M | 320 F (mild focal subscapular fibroplasia in adrenal gland) | | | |
| | | | Bd Wt | 320 | | | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **41** | Mouse (B6C3F1) | 6 mo (F) | Resp | 100 | | | U.S. Army 1984c | |
| | | | Cardio | 100 | | | | |
| | | | Gastro | 100 | | | | |
| | | | Hemato | 100 | | | | |
| | | | Musc/skel | 100 | | | | |
| | | | Hepatic | 100 | | | | |
| | | | Renal | 100 | | | | |
| | | | Endocr | 100 | | | | |
| | | | Ocular | 100 | | | | |
| **42** | Dog (NS) | 90 d (F) | Resp | 10 | | | U.S. Navy 1974a | |
| | | | Cardio | 10 | | | | |
| | | | Hemato | 10 | | | | |
| | | | Hepatic | 10 | | | | |
| | | | Renal | 10 | | | | |
| | | | Endocr | 10 | | | | |
| | | | Ocular | 10 | | | | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **43** | Dog (NS) | 6 wk 6 d/wk (C) | Resp | 50 F | | | von Oettingen et al. 1949 | |
| | | | Cardio | 50 F | | | | |
| | | | Hemato | 50 F | | | | |
| | | | Hepatic | 50 F | | | | |
| | | | Renal | 50 F | | | | |
| | | | Endocr | 50 F | | | | |
| | | | Bd Wt | | | 50 F (unspecified weight loss) | | |
| **Immuno/ Lymphoret** | | | | | | | | |
| **44** | Rat (Fischer- 344) | 7 d/wk 90 d (GW) | | 15 | | | U.S. Army 2006 | |
| **Neurological** | | | | | | | | |
| **45** | Monkey (Cynomolgus) | 90 d 7 d/wk (GW) | | 1 | | 10 (convulsions and seizures) | U.S. Navy 1974b | |
| **46** | Rat Fischer 344 | 13 wk (F) | | 30 | 100 (hyperreactive to approach) | | Levine et al. 1981 | |
| **47** | Rat (Fischer 344) | 10 wk (F) | | 30 | 100 (hyperreactive to approach) | | Levine et al. 1990 | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **48** | Rat (Fischer 344) | 25 wk (F) | | 8 | | 40 (tremor, convulsions, hyperreactive) | U.S. Army 1983a | |
| **49** | Rat (Sprague-Dawley) | 30 d (GW) | | 10 M | | | U.S. Army 1985b | testing conducted 24 hours after dose administration |
| **50** | Rat (Fischer- 344) | 7 d/wk 90 d (GW) | | 4[c] | | 8 (tremors and convulsions) | U.S. Army 2006 | |
| **51** | Rat (NS) | 90 d (F) | | 15 | | 25 (convulsions, hyperirritability and fighting) | von Oettingen et al. 1949 | |
| **52** | Rat (NS) | 10 wk (F) | | 15 | | 50 (hyperirritability and convulsions) | von Oettingen et al. 1949 | |
| **53** | Mouse (B6C3F1) | 90 d (F) | | 160 M | 320 M (hyperactivity and/or nervousness) | | U.S. Army 1980b | |
| **54** | Dog (NS) | 6 wk 6 d/wk (C) | | | | 50 F (hyperirritability and convulsions) | von Oettingen et al. 1949 | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **Reproductive** | | | | | | | | |
| **55** | Rat (Fischer 344) | 3-13 wk (F) | | 100 | | | Levine et al. 1990 | |
| **56** | Rat (Fischer 344) | 2 generation; 13 wk pre-mating, mating, gestation, & lactat. (F) | | 50 | | | U.S. Army 1980b | |
| **57** | Rat (Fischer 344) | 15 wk (F) | | 16 M | 50 M (decreased fertility) | | U.S. Army 1980b | Decreased fertility may be due to RDX-effect on general well-being of males |
| **58** | Rat (Fischer 344) | 6 mo (F) | | 8 M<br>40 F | 40 M (spermatic granuloma in prostate) | | U.S. Army 1983a | |
| **Developmental** | | | | | | | | |
| **59** | Rat (Fischer 344) | 2 generation; 13 wk pre-mating, mating, gestation, & lactat. (F) | | 5 | 16 (decrease in F2 pup body weight) | 50 (increase in number of stillbirths; decrease in pup survival in F1) | U.S. Army 1980b | |
| **60** | Rabbit (NS) | Gd 7-29 (GW) | | 20 | | | U.S. Army 1980b | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **CHRONIC EXPOSURE** | | | | | | | | |
| **Death** | | | | | | | | |
| **61** | Rat (Fischer 344) | 2 yr (F) | | | | 40 M (88% died) | U.S. Army 1983a | |
| **Systemic** | | | | | | | | |
| **62** | Rat (Fischer 344) | 1 & 2 yr (F) | Resp | 40 | | | U.S. Army 1983a | |
| | | | Cardio | 40 | | | | |
| | | | Gastro | 40 | | | | |
| | | | Hemato | 8 | 40 (decreases in hematocrit, hemoglobin, and erythrocyte levels; splenic extramedullary hematopoiesis) | | | |
| | | | Musc/skel | 40 | | | | |
| | | | Hepatic | 8 | 40 (hepatomegaly, decreased serum trigylcerides and cholesterol) | | | |
| | | | Renal | 8 | 40 (renal papillary necrosis with increased BUN) | | | |
| | | | Endocr | 40 | | | | |
| | | | Ocular | 8 F | 40 F (cataracts) | | | |
| | | | Bd Wt | 8 | | 40 M (20-30% decrease in body weight gain) | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **63** | Rat (Sprague-Dawley) | 2 yr (F) | Resp | 10 | | | U.S. Navy 1976 | |
| | | | Cardio | 10 | | | | |
| | | | Gastro | 10 | | | | |
| | | | Hemato | 10 | | | | |
| | | | Hepatic | 10 | | | | |
| | | | Renal | 10 | | | | |
| | | | Endocr | 10 | | | | |

Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **64** | Mouse (B6C3F1) | 1 & 2 yr (F) | Resp | 100 | | | U.S. Army 1984c | |
| | | | Cardio | 35 | 100 (increased relative heart weight) | | | |
| | | | Gastro | 100 | | | | |
| | | | Hemato | 100 | | | | |
| | | | Musc/skel | 100 | | | | |
| | | | Hepatic | 7 | 35 F (increased serum cholesterol levels) | | | |
| | | | Renal | 35 | 100 (increased relative kidney weights and reversible cytoplasmic vacuolization) | | | |
| | | | Ocular | 100 | | | | |
| | | | Bd Wt | 100 | | | | |
| **Neurological** | | | | | | | | |
| **65** | Rat (Fischer 344) | 1 & 2 yr (F) | | 8[d] | | 40 (tremors, convulsions; hyperresponsive to stimuli) | U.S. Army 1983a | |
| **Reproductive** | | | | | | | | |
| **66** | Rat (Fischer 344) | 1 & 2 yr (F) | | 8 M<br>40 F | 40 M (testicular degeneration) | | U.S. Army 1983a | |

**Table 3-1 Levels of Significant Exposure to RDX - Oral (continued)**

| Key to Figure[a] | Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL (mg/kg/day) | LOAEL Less Serious (mg/kg/day) | LOAEL Serious (mg/kg/day) | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|---|
| **67** | Mouse (B6C3F1) | 1 & 2 yr (F) | | 100 M<br>100 F | | | U.S. Army 1984c | |
| **Cancer** | | | | | | | | |
| **68** | Mouse (B6C3F1) | 1 & 2 yr (F) | | | | 35 F (CEL: hepatocellular carcinomas and adenomas) | U.S. Army 1984c | |

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

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.2 mg/kg/day based on a PBPK model predicted internal dose metric (peak brain RDX concentration) of the NOAEL dose; a human equivalent dose (HED) of the NOAEL was also estimated using a PBPK model. The NOAELHED of 6.45 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustments and 10 for human variability).

c Used to derive an intermediate-duration oral MRL of 0.1 mg/kg/day based on a BMDL10 estimated using using a PBPK model predicted internal dose metric (peak brain RDX concentration); a human equivalent dose of the BMDL10 was also predicted using a PBPK model. The BMDLHED of 4.1308 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustment and 10 for human variability).

d Used to derive a chronic-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on a PBPK model predicted internal dose metric (peak brain RDX concentration) of the NOAEL dose; a human equivalent dose (HED) of the NOAEL was also estimated using a PBPK model. The NOAELHED of 4.223 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustments and 10 for human variability).

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Metab = metabolic; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to RDX - Oral

Acute (≤14 days)

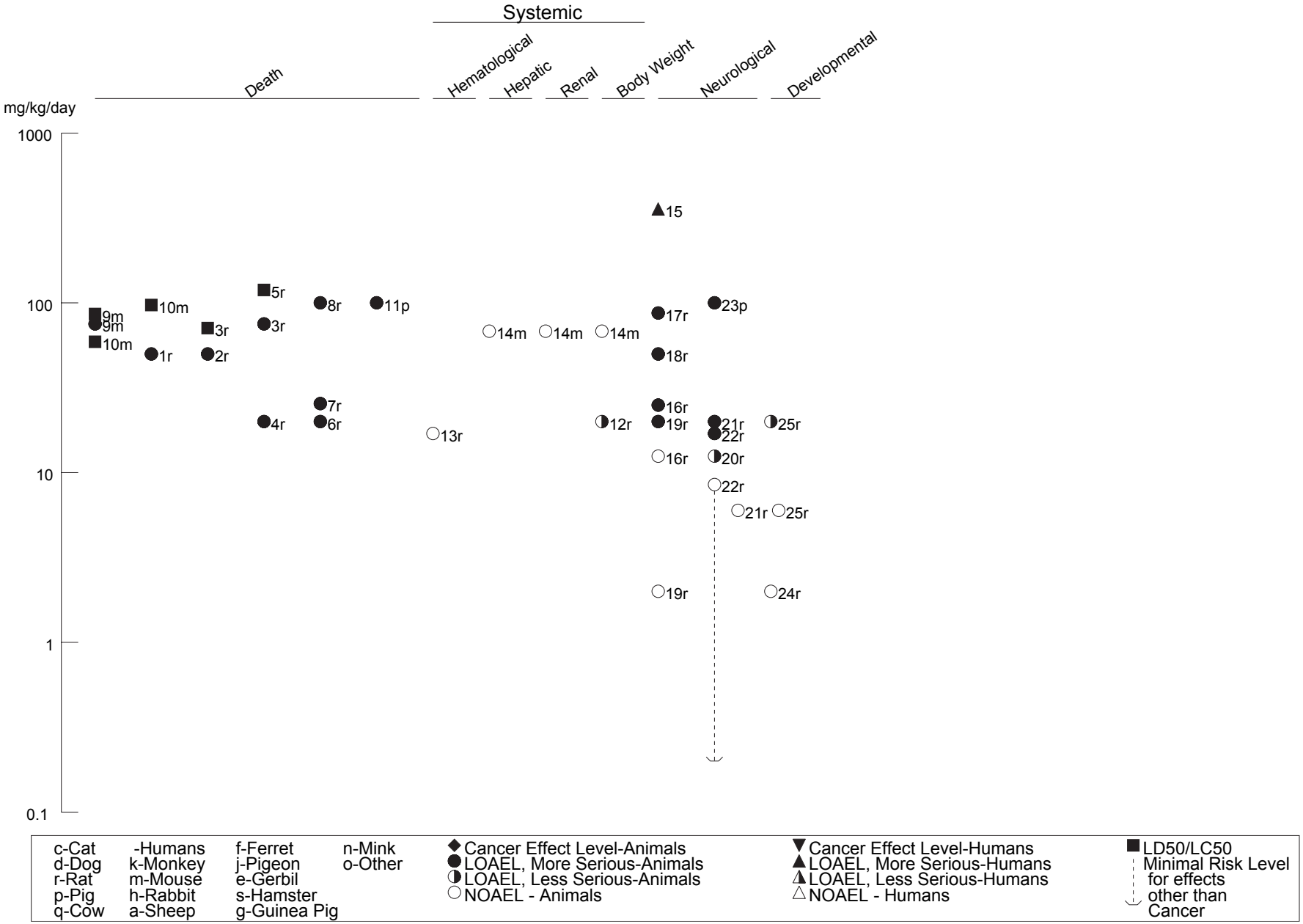


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)

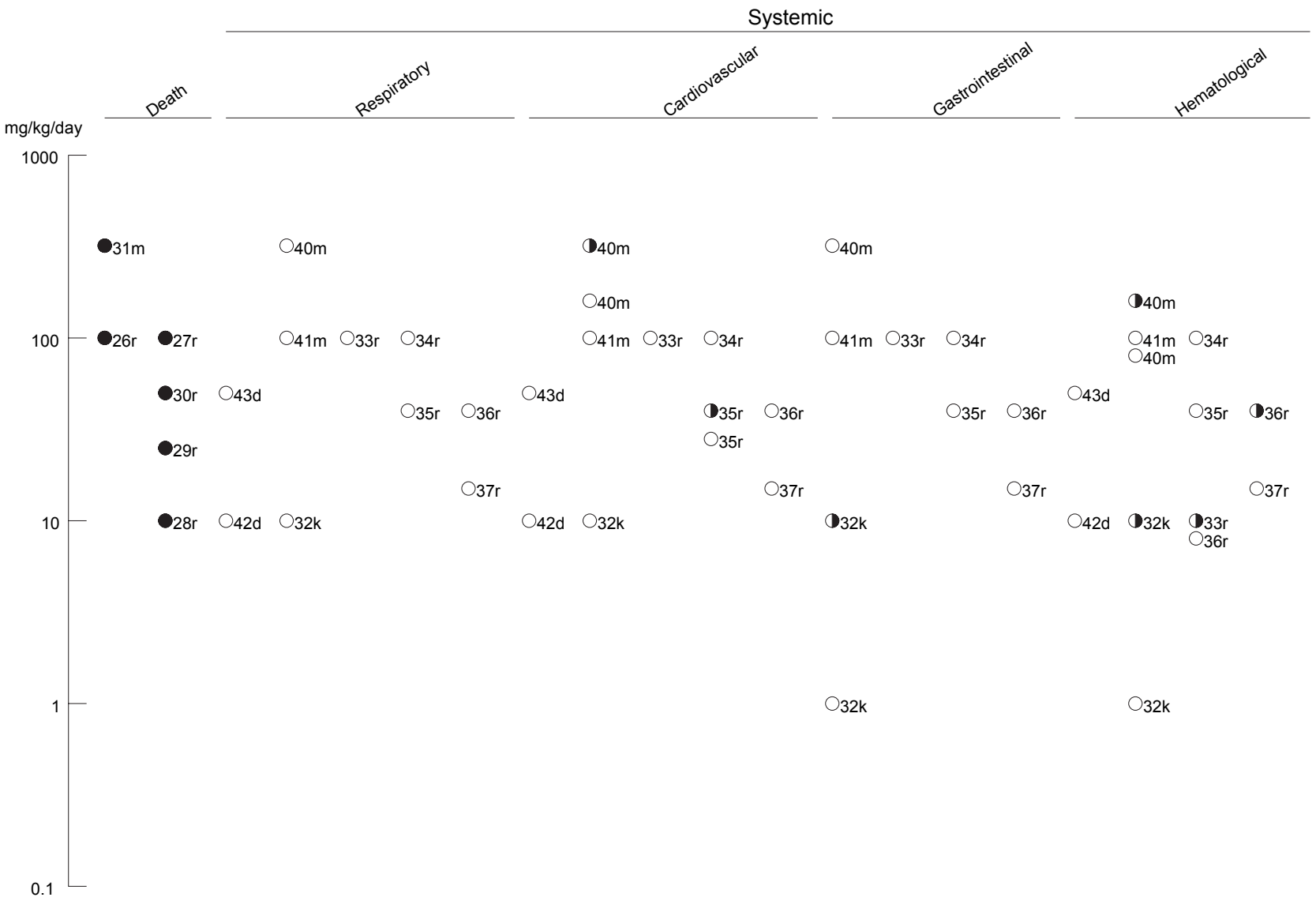


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)

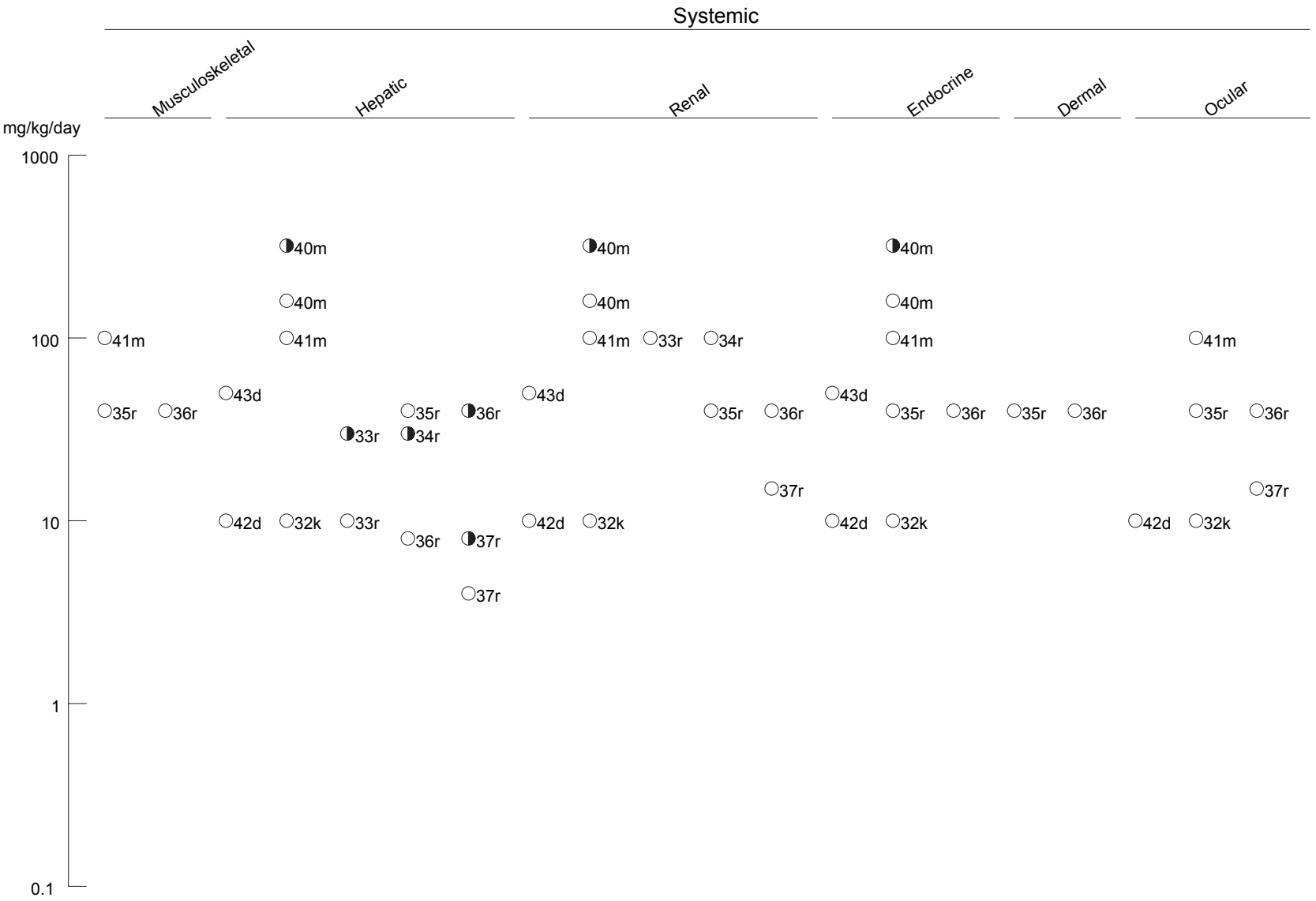
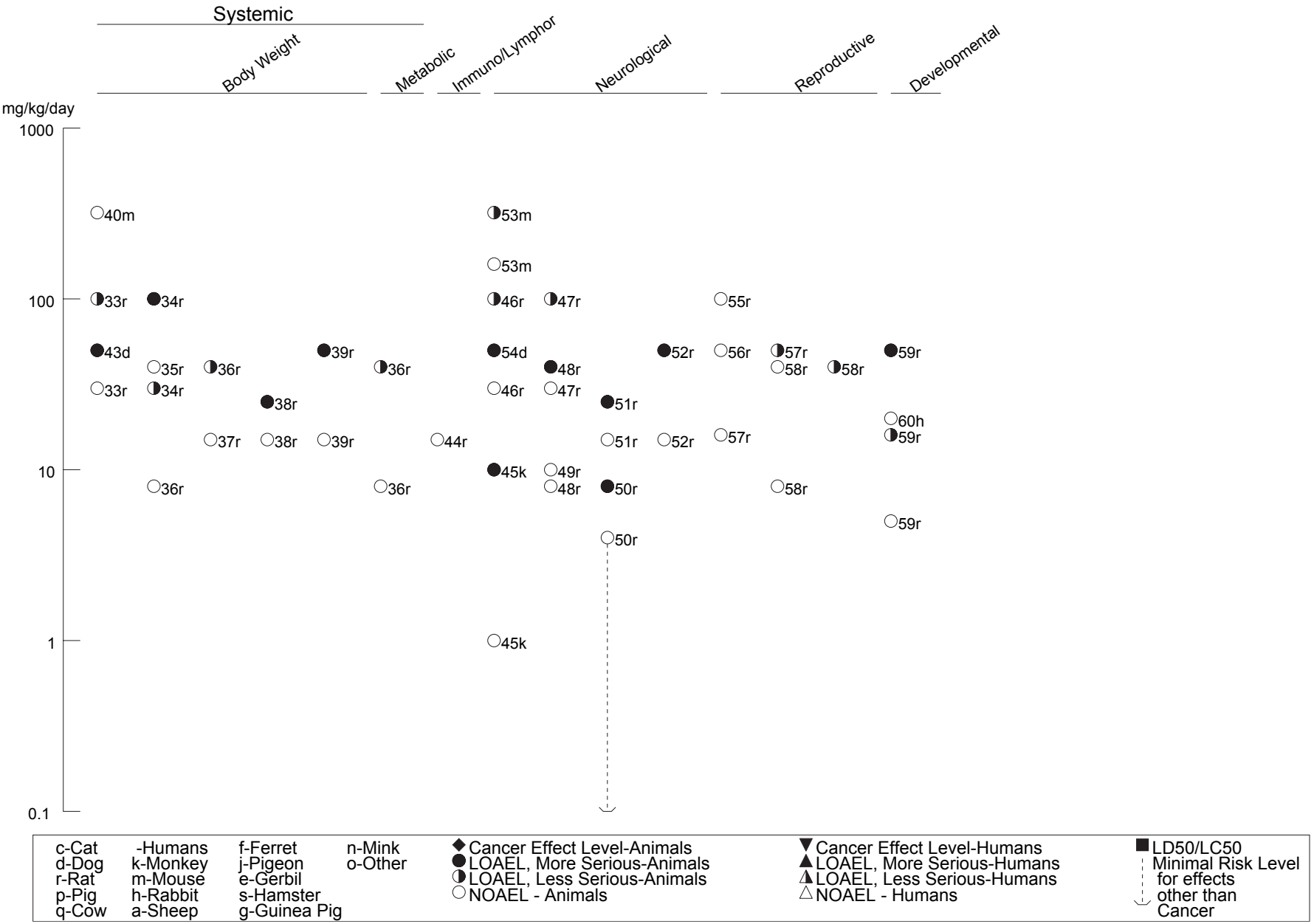


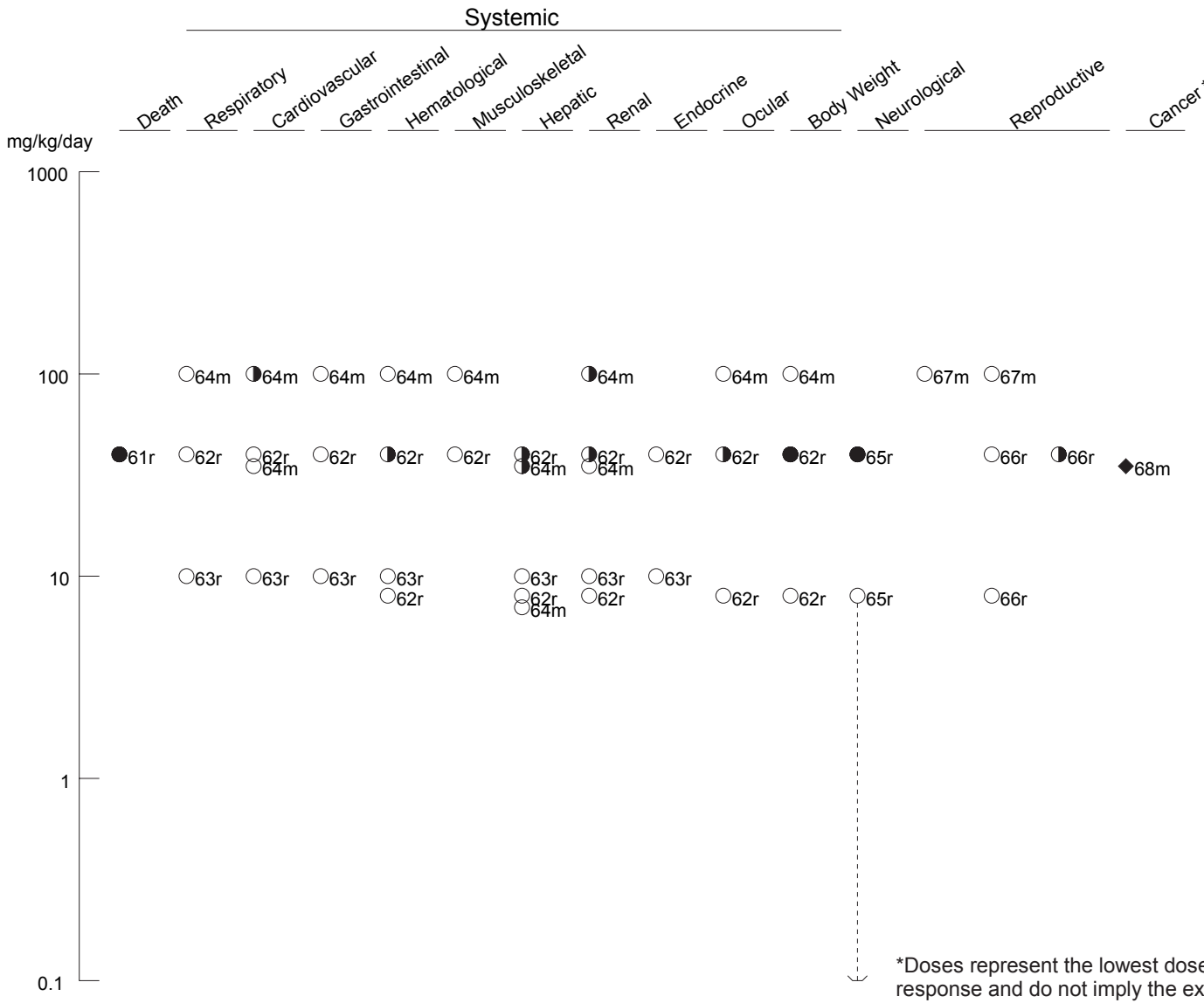
Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)



## Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Chronic (≥365 days)




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

c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

### 3.2.2.2 Systemic Effects

No studies were located regarding respiratory, musculoskeletal, dermal, or ocular effects in humans after acute oral exposure to RDX. No studies were located regarding systemic effects in humans after intermediate or chronic oral exposure to RDX. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

**Respiratory Effects.** Adverse respiratory effects were not observed in animals following acute, intermediate, or chronic exposure. An acute-duration study in 3 anesthetized dogs showed no significant changes in breathing rate when 15 mg/kg RDX was administered by gavage (von Oettingen et al. 1949). No histopathology was seen in the lungs, trachea, or bronchi of rats exposed for 3–13 weeks to 100 mg/kg/day of RDX in the diet (Levine et al. 1990), 40 mg/kg/day RDX in the diet for 90 days (U.S. Army 1980b), or 15 mg/kg/day via gavage for 90 days (U.S. Army 2006). Similarly, no histopathological alterations were observed in the respiratory system of mice exposed to 100 or 320 mg/kg/day in the diet for 3 or 6 months (U.S. Army 1980b, 1984c), dogs exposed to 10 mg/kg/day in the diet for 90 days (U.S. Navy 1974a) or 50 mg/kg via capsules 6 days/week for 6 weeks (von Oettingen et al. 1949), or monkeys administered 10 mg/kg/day via gavage for 90 days (U.S. Navy 1974b). Chronic-duration studies also revealed no histopathology in rats (U.S. Army 1983a; U.S. Navy 1976) or mice (U.S. Army 1984c).

**Cardiovascular Effects.** Sinusoidal tachycardia was observed in five men who accidentally ingested 37–250 mg/kg RDX (Küçükardalĭ et al. 2003).

Few, if any, changes were observed in cardiovascular parameters measured in animals exposed to RDX. An acute-duration study in 3 anesthetized dogs showed no significant changes in heart rate when 15 mg/kg RDX was administered by gavage (von Oettingen et al. 1949). Intermediate-duration studies revealed no histopathology in the heart of rats exposed to 15–100 mg/kg/day of RDX (Levine et al. 1981; U.S. Army 2006). Slight myocardial degeneration was observed in rats exposed to 40 mg/kg/day and mice exposed to 320 mg/kg/day in the diet for 90 days (U.S. Army 1980b). No pathology was seen in the hearts of dogs (U.S. Navy 1974a; von Oettingen et al. 1949) or monkeys (U.S. Navy 1974b) exposed to RDX for intermediate periods. Hyaline degeneration of the heart muscles was observed in rats following intermediate exposure to 50 mg/kg/day of RDX (Sunderman 1944). Chronic exposure produced no cardiac histopathology in rats (U.S. Army 1983a; U.S. Navy 1976), but it increased relative heart weights in mice (U.S. Army 1984c).

**Gastrointestinal Effects.** Humans who accidentally or intentionally consumed unknown levels of RDX for an acute period had nausea, vomiting, and abdominal pain (Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003). In three of five cases in which men accidentally ingested 37–250 mg/kg, an endoscopic examination conducted 3 days after exposure revealed erosive gastroduodenitis (Küçükardalĭ et al. 2003).

Vomiting was reported in dogs acutely exposed to 100 and 300 mg/kg/day RDX (Sunderman 1944). Vomiting was also observed in five of six monkeys administered via gavage 10 mg/kg/day for 90 days, compared to one of six in the control group (U.S. Navy 1974b). There were 15 episodes of vomiting (excluding vomiting, which occurred during the gavage procedure) in this group compared to 1 episode in the control group. In monkeys administered 1 mg/kg/day, two of six animals (three episodes) vomited; one other animal in this group vomited only during the gavage procedure. Following intermediate exposure of rats to 50 mg/kg/day RDX, mild congestion of the intestines was reported (Sunderman 1944). No histopathology was seen in the stomachs or intestines of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a), mice (U.S. Army 1980b, 1984c), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), or monkeys (U.S. Navy 1974b). Chronic exposure also did not produce histopathological alterations in rats (U.S. Army 1983a; U.S. Navy 1976) or mice (U.S. Army 1984c).

**Hematological Effects.** Humans who accidentally consumed unknown levels of RDX for an acute duration generally had normal blood counts (Ketel and Hughes 1972; Woody et al. 1986). Temporary decreased hematocrit and leukocytosis were reported in a study of six men who consumed C-4 containing RDX (Stone et al. 1969). Similarly, leukocytosis and methemoglobinemia were noted in a report of five men accidentally ingesting 37–250 mg/kg RDX (Küçükardalĭ et al. 2003).

Decreased hemoglobin and erythrocyte levels, increased platelet counts, and splenic extramedullary hematopoiesis were observed in male rats exposed to 40 mg/kg/day RDX in the diet for 6 months (U.S. Army 1983a). However, oral doses of 15 mg/kg/day (administered via gavage) (U.S. Army 2006) or 40 mg/kg/day (administered via the diet) (U.S. Army 1980b) for 13 weeks did not result in significant hematological effects. Similarly, decreased hemoglobin and erythrocyte levels were observed in mice exposed to 160 mg/kg/day for 90 days (U.S. Army 1980b). No significant hematological effects were found in mice exposed to 100 mg/kg/day for 6 months (U.S. Army 1984c) and dogs exposed to 50 mg/kg/day for 6 weeks (von Oettingen et al. 1949). Species differences in hematological responses to RDX may relate to differences in their activity of erythrocyte methemoglobin reductase (Rockwood et al.

2003; Smith and Beutler 1966). Slight, but statistically significant, increases in the number of leukocytes were observed in rats exposed to ≥10 mg/kg/day for 13 weeks (Levine et al. 1981). Necrotic and degenerative megakaryocytes were observed in the bone marrow of monkeys given 10 mg/kg/day of RDX for 90 days (U.S. Navy 1974b). Chronic administration of 40 mg/kg/day of RDX in the diet for 1–2 years produced decreased hematocrit, hemoglobin, and erythrocytes in rats; the effects were not considered biologically significant and there were no compensatory responses (U.S. Army 1983a). Significant increases in platelet levels were also observed at 40 mg/kg/day (U.S. Army 1983a). No significant hematological effects were observed in mice chronically exposed to 100 mg/kg/day (U.S. Army 1984c).

**Musculoskeletal Effects.** No histopathological alterations were observed in muscle or skeletal tissue of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a), mice (U.S. Army 1980b, 1984c), or dogs (U.S. Navy 1974a) exposed for intermediate periods. Muscles and bones were also normal in rats (U.S. Army 1983a; U.S. Navy 1976) and mice (U.S. Army 1984c) exposed for chronic periods.

**Hepatic Effects.** Slightly elevated serum aspartate aminotransferase and/or alanine aminotransferase (Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969) were observed in humans ingesting unknown levels of RDX after using C-4 cooking fuel for an acute duration; other studies reported normal liver enzyme levels (Ketel and Hughes 1972). Liver biopsies were normal (Stone et al. 1969).

Minor adverse hepatic effects have been noted in some animal studies. Slight decreases in alanine aminotransferase levels were observed in rats exposed to 40 mg/kg/day for 26 weeks (U.S. Army 1983a). Decreases in serum triglyceride levels were noted in rats exposed to ≥10 mg/kg/day RDX for 13 weeks (Levine et al. 1981, 1990), decreases in serum cholesterol were observed in male rats administered ≥8 mg/kg/day for 90 days (U.S. Army 2006), and decreases in serum triglycerides and cholesterol were observed in rats exposed to 40 mg/kg/day for 6 months to 2 years (U.S. Army 1983a). Increases in serum cholesterol were also observed in female mice exposed to 35 or 100 mg/kg/day for 1–2 years (U.S. Army 1984c). Increases in liver weight have been observed in rats exposed to 30 or 100 mg/kg/day (Levine et al. 1981, 1990) and mice exposed to 100 or 320 mg/kg/day (U.S. Army 1980b, 1984c); hepatomegaly was observed in rats exposed to 40 mg/kg/day (U.S. Army 1983a). However, most studies did not find histological alterations in the livers of rats (Levine et al. 1981; U.S. Army 1983a), mice (U.S. Army 1984c), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), white-footed mice (U.S. Army 1999), or monkeys (U.S. Navy 1974b). Two studies did find histological effects; hepatocellular vacuolization was observed in mice exposed to 320 mg/kg/day for 90 days (U.S. Army 1980b) and fatty degeneration was

observed in rats exposed to 50 mg/kg/day for 78 days (Sunderman 1944). Although the alterations in serum clinical chemistry parameters may be indicative of minor changes in liver function, the lack of histological damage at similar or higher doses suggests that the liver may not be a sensitive target of RDX toxicity and the alterations may not be biologically significant.

**Renal Effects.** Humans who accidentally consumed unknown levels of RDX for an acute duration showed no (Woody et al. 1986) or only slight (Ketel and Hughes 1972; Stone et al. 1969) changes in renal function parameters. Proteinuria and glucosuria were observed in men after accidental ingestion of RDX (Küçükardalĭ et al. 2003; Merrill 1968).

Few adverse renal effects were reported in animals. No histopathological alterations were observed in the kidneys from white-footed mice following 14-day dietary exposure (U.S. Army 1999) or from rats following intermediate exposure periods (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a, 2006). Normal kidney parameters were also observed in dogs (U.S. Navy 1974a; von Oettingen et al. 1949) and monkeys (U.S. Navy 1974b). In contrast, mild tubular nephrosis was reported in mice given high doses (320 mg/kg/day) in the food for 13 weeks, but was not seen at lower doses (160 mg/kg/day) (U.S. Army 1980b). Following chronic exposure to 40 mg/kg/day of RDX in food, renal papillary necrosis and elevated blood urea nitrogen levels were observed in rats (U.S. Army 1983a); these effects were not observed at 8 mg/kg/day. Other studies showed normal renal parameters in rats at lower levels (10 mg/kg/day) (U.S. Navy 1976). Increased kidney weights, but no other signs of kidney toxicity, were observed in mice chronically exposed to 100 mg/kg/day (U.S. Army 1984c).

**Endocrine Effects.** No histopathological alterations were observed in the adrenal glands of rats (U.S. Army 1980b, 1983a; U.S. Navy 1976), mice (U.S. Army 1984c), dogs (U.S. Navy 1974a), or monkeys (U.S. Navy 1974b) exposed for intermediate periods. One study (U.S. Army 1980b) observed mild focal subscapular fibroplasia in the adrenal glands of female mice exposed to 320 mg/kg/day RDX for 90 days.

**Dermal Effects.** No significant skin lesions were seen in rats (U.S. Army 1980b, 1983a) exposed for intermediate periods to RDX in the food.

**Ocular Effects.** No significant ophthalmologic alterations were observed in rats administered 15 mg/kg/day via gavage for 90 days (U.S. Army 2006). Female rats exposed to 40 mg/kg/day of RDX in their food for 2 years had cataracts (U.S. Army 1983a), but this was not seen in the male rats in this

study, in male or female rats exposed to 40 mg/kg/day in another study (U.S. Army 1980b), or in mice exposed to a higher level (100 mg/kg/day) (U.S. Army 1984c).

**Body Weight Effects.** Weight loss or lack of weight gain of >10% was seen in rats fed 25–40 mg/kg/day (Levine et al. 1981, 1990; von Oettingen et al. 1949) and dogs fed 50 mg/kg/day (von Oettingen et al. 1949) for an intermediate duration, and in rats receiving 40 mg/kg/day RDX (U.S. Army 1983a) and mice receiving 100 mg/kg/day (U.S. Army 1984c) for a chronic period.

**Metabolic Effects.** Hyperglycemia, hypokalemia, and metabolic acidosis with anion gap were observed in men accidentally ingesting 37–250 mg/kg RDX (Küçükardalĭ et al. 2003). In rats exposed to 40 mg/kg/day RDX in the diet for 13–78 weeks, significant decreases in blood glucose levels were observed (U.S. Army 1983a).

### 3.2.2.3 Immunological and Lymphoreticular Effects

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

No studies were located regarding immunological effects in animals after acute oral exposure to RDX. Studies of intermediate duration (6–13 weeks) failed to reveal any marked pathological alterations in the spleen, thymus, and/or lymph nodes in rats (Levine et al. 1990; U.S. Army 1980b), mice (U.S. Army 1980b), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), or monkeys (U.S. Navy 1974b). No significant alterations in spleen and thymus organ weights or cellularity, or in the proportion of cell surface markers were observed in rats exposed to 15 mg/kg/day for 90 days (U.S. Army 2006). The NOAEL value from the U.S. Army (2006) is recorded in Table 3-1 and plotted in Figure 3-1; NOAEL values for studies only examining potential histopathological alterations in lymphoreticular organs were not listed.

### 3.2.2.4 Neurological Effects

The available studies have identified the nervous system as a target system in humans following oral exposure to RDX. Numerous case reports are available that describe seizures in men (Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969) and in one child (Woody et al. 1986) after accidental consumption of unknown quantities of RDX for acute periods and in men intentionally chewing on C-4 (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995). The RDX was almost always mixed with other components in the form of the

explosive C-4, which is 91% RDX (mixed with polyisobutylene, motor oil, and di-(2-ethylhexyl) sebacate). In most of the cases, RDX intakes were not known; Stone et al. (1969) reported doses (357 and 2,571 mg/kg) for two cases. In a report of five cases (Küçükardalĩ et al. 2003), repetitive tonic-clonic convulsions were first observed 4–16 hours after RDX exposure; in one case, convulsions were observed for 3 days, although the frequency and duration gradually decreased with time. Most studies reported that recovery occurred within a few days or weeks. Accompanying complaints included disorientation, nausea, restlessness, muscle twitching, lethargy, and hyperactive deep tendon reflexes. In most cases, no other neurological evaluations were performed. Küçükardalĩ et al. (2003) noted abnormalities in electroencephalograms (EEG) in three of the five cases. In the case reported by Harrell-Bruder and Hutchins (1995), no EEG abnormalities were found. No intermediate- or chronic-duration exposure data have been reported for humans.

Animal studies have also shown that the nervous system is a target system following oral exposure to RDX. Seizures were observed in rats receiving a single gavage dose of ≥25 mg/kg (U.S. Army 2006; Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977), deer mice receiving a gavage dose of 136 mg/kg (Smith et al. 2007), and miniature swine receiving a single gavage dose of ≥10 mg/kg (JHU/U.S. Army 2006; Schneider et al. 1977). Seizures were also observed in 20% of rats administered a single dose of 12.5 mg/kg/day; however, the incidence was not significantly different from controls (Burdette et al. 1988). Following administration of a single dose of ≥25 mg/kg RDX, seizures and convulsions typically occur within 3 hours (U.S. Army 2006; Burdette et al. 1988). Convulsions and hyperactivity were also noted in "several" surviving rat dams administered 20 mg/kg/day by gavage during gestation days 6–15 (U.S. Army 1986d); incidence data were not provided. Hyperactivity was observed in approximately 70% of rat dams administered 20 mg/kg/day RDX via gavage on gestation days 6–19 (U.S. Army 1980b). In a 14-day exposure study, tremors and convulsions were observed in rats receiving gavage doses of ≥17 mg/kg/day; no marked neurological effects were noted at 8.5 mg/kg/day (U.S. Army 2006). In neurobehavioral tests, decreases in motor activity, hindlimb splay, taste aversion to saccharine, response rate in scheduled-controlled behavior tests, auditory startle amplitude, and increases in startle latency were observed in rats receiving a single gavage dose of ≥12.5 mg/kg/day (U.S. Army 1985b).

Seizures and convulsions have also been observed in rats, monkeys, and dogs exposed to RDX for intermediate or chronic durations. Seizures and convulsions were observed in rats administered gavage doses ≥8 mg/kg/day for 90 days (U.S. Army 2006), in rats exposed to dietary RDX at doses of ≥25 mg/kg/day (Sunderman 1944; U.S. Army 1983a; von Oettingen et al. 1949), dogs exposed to 50 mg/kg/day via a capsule (von Oettingen et al. 1949), and monkeys administered 10 mg/kg/day via

gavage (U.S. Navy 1974b). Few studies have reported the time course of the convulsions/seizures. In the U.S. Army (2006) study, convulsions/seizures were observed at the beginning of the study in rats administered 12 or 15 mg/kg/day and were seen throughout the study; convulsions/seizures were also observed at 8 and 10 mg/kg/day, however, the investigators did not note when the convulsions/seizures first occurred for these dose levels. In a chronic dietary study, seizures and convulsions were first observed after 26 weeks of exposure to 40 mg/kg/day (U.S. Army 1983a). In monkeys, the effects were typically observed after 34–57 doses, although effects were also seen in some monkeys after the 2$^{nd}$ or 12$^{th}$ dose, and did not occur on a regular basis (U.S. Navy 1974b). Other overt neurological signs observed following intermediate or chronic exposure include hyperactivity, hyperreactivity, increased arousal, and increased fighting in rats exposed to gavage doses of ≥10 mg/kg/day (U.S. Army 2006), in rats exposed to ≥25 mg/kg/day in the diet (Levine et al. 1990; U.S. Army et al. 1983a; von Oettingen et al. 1949), and dogs administered capsules containing 50 mg/kg/day (von Oettingen et al. 1949). The highest NOAELs for overt neurological effects were 4 mg/kg/day in rats receiving gavage doses (U.S. Army 2006), 15 mg/kg/day in rats exposed via the diet (von Oettingen et al. 1949), and 1 mg/kg/day in monkeys receiving gavage doses (U.S. Navy 1974b). Neurobehavioral performance was assessed in rats receiving gavage doses of RDX for 30 or 90 days. No significant alterations were observed in motor activity, flavor aversion, scheduled-controlled behavior, or acoustic startle in rats administered 10 mg/kg/day for 15 or 30 days (U.S. Army 1985b). No RDX-related alterations in foot splay, front limb grip strength, or response to stimuli were found in rats administered 15 mg/kg/day for 90 days (U.S. Army 2006). No significant histological alterations have been found in the brain (U.S. Army 1983a, 2006).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

#### 3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to RDX.

Toxicity studies lasting 13 weeks showed no pathological changes in the gonads or uteri of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a) or mice (U.S. Army 1980b, 1984c) exposed to RDX. No functional tests were performed. One study did report spermatic granulomas in the prostates of rats exposed to 40 mg/kg/day for 6 months (U.S. Army 1983a); this effect was not observed in rats exposed after 1 or 2 years of exposure (U.S. Army 1983a). This study (U.S. Army 1983a) also reported an

increase in the incidence of testicular degeneration in rats exposed to 40 mg/kg/day for 6 months (3/10, not statistically significant) or 1 year (4/10), but not after 2 years (0/4).

Histological examinations of rats exposed to ≥1.5 mg/kg/day in the feed for 2 years revealed suppurative inflammation in the prostate (U.S. Army 1983a). The prostate effects were predominantly observed in rats dying early and may have been secondary to a bacterial infection of the urinary tract. Urinary bladder distention and cystitis were observed in rats exposed to 40 mg/kg/day for 1 or 2 years. Testicular degeneration was observed in rats exposed to 40 mg/kg/day for 1 year (U.S. Army 1983a); a nonstatistically significant increase in testicular degeneration was also observed in mice exposed to ≥35 mg/kg/day for 1–2 years (U.S. Army 1984c). No significant histological alterations have been observed in the ovaries or uterus of rats (U.S. Army 1983a) or mice (U.S. Army 1984c) chronically exposed to RDX.

Two studies examined reproductive function. In a two-generation study, no significant alterations in reproduction were observed in the $F_0$ and $F_1$ rats exposed to 16 mg/kg/day in the diet (U.S. Army 1980b). At 50 mg/kg/day, nonstatistically significant decreases in fertility were observed in the $F_0$ generation. In a dominant lethality assay (U.S. Army 1980b), decreases in fertility were observed in male rats exposed to 50 mg/kg/day for 15 weeks prior to mating with unexposed females; however, the investigators noted that this effect may have been secondary to the impaired well-being of the males. The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

#### 3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to RDX.

There are two available developmental studies in rats (exposed for 9 or 13 days during gestation) that are inconclusive because of excessive maternal toxicity at the high dose (20 mg/kg/day). In one study, no excessive gross, visceral, or skeletal anomalies were found in fetuses when the dams were exposed to 2 mg/kg/day of RDX (U.S. Army 1980b). High maternal lethality, decreased maternal body weights, and adverse maternal neurological effects precluded judgment regarding fetal toxicity at 20 mg/kg/day. The other rat study (U.S. Army 1986d) also showed high maternal toxicity (increased mortality and seizures) at 20 mg/kg/day. These investigators also reported a significant decrease in fetal weights and lengths at ≥2 mg/kg/day when data were analyzed on an individual basis rather than a litter basis. However, it

appears that there was an overlap in the standard deviations for the fetal body weight and length values; when analyzed on a litter basis, decreases in fetal weights and lengths were only significant at the 20 mg/kg/day dose level. In contrast to rats, rabbits (exposed for 22 days during gestation) showed no adverse fetal or maternal effects at 20 mg/kg/day (U.S. Army 1980b). In a two-generation reproduction study, an increase in the number of stillbirths, a decrease in the number of pups per litter at birth, and a decrease in the number of live litters at weeks 7, 14, and 21 were observed in the $F_1$ offspring of rats exposed to 50 mg/kg/day (a dose that also resulted in increased maternal deaths and decreased feed consumption) (U.S. Army 1980b). In the $F_2$ generation, a decrease in terminal body weights and an increase in renal tubular epithelial-lined cysts were observed at 16 mg/kg/day. Similar cysts were observed in $F_2$ pups exposed to 0 or 5 mg/kg/day. The highest NOAEL values and all reliable LOAEL values for developmental effects for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

#### 3.2.2.7 Cancer

No studies were located regarding cancer in humans after oral exposure to RDX.

RDX was not found to be carcinogenic when fed to F344 rats (U.S. Army 1983a) or Sprague-Dawley rats (U.S. Navy 1976) for at least 1 year. Adequate doses, numbers of animals, and survival rates were achieved for both of these studies. Only female $B6C3F_1$ mice showed an increased incidence of combined hepatocellular adenomas and carcinomas when compared to concurrent or historical controls (U.S. Army 1984c). However, a re-evaluation of these data using revised diagnostic criteria resulted in a reclassification of several hepatocellular adenomas as foci of cytoplasmic alterations (Parker et al. 2006). As noted in the abstract of the Parker et al. (2006) paper, the combined incidence of hepatocellular adenomas and carcinomas was significantly increased (no information regarding statistical analysis was presented in the paper) in the 35 mg/kg/day group, but not in the 100 mg/kg/day group. The investigators noted that the combined incidence of hepatocellular adenomas and carcinomas in the 35 mg/kg/day group (10/64, 16%) was within the range of published historical control data (0–21%) and suggested that the study provided equivocal evidence of a carcinogenic effect. The 35 mg/kg/day dose is listed as a cancer effect level (CEL) in Table 3-1 and Figure 3-1. The lifetime average doses that would result in risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are 0.9, 0.09, 0.009, and 0.0009 mg/kg/day, respectively, as indicated in Figure 3-1.

### 3.2.3 Dermal Exposure

#### 3.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to RDX.

Deaths were observed in rabbits receiving repeated dermal applications of 37.5 mg/kg/day RDX in cyclohexanone (1/6 deaths) or 27 mg/kg/day RDX in acetone (2/6) deaths; no gross pathological effects were seen (U.S. Army 1974). Because of the lack of data presented, it is difficult to determine whether RDX alone was responsible for the deaths reported in this study.

#### 3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans after dermal exposure to RDX. Two older studies of dermal and ocular effects were located for humans following dermal exposure to RDX.

One animal study examined the potential systemic toxicity of RDX following a single dermal application (U.S. Army 1974); however, no details were provided regarding the "pathological examination"; thus, NOAELs for systemic effects were not presented for this study. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-2.

**Respiratory Effects.** No alterations were noted in the respiratory rates of dogs following single or multiple dermal exposures to RDX in dimethyl sulfoxide (DMSO) (U.S. Army 1974).

**Cardiovascular Effects.** No adverse effects were seen on blood pressure, heart rate, or electrocardiograms of dogs dermally exposed to a single application or repeated exposure (5 days/week for 4 weeks) of 289 mg/kg RDX in DMSO (U.S. Army 1974). No lesions were seen in the hearts of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

**Gastrointestinal Effects.** Necropsy did not reveal any lesions in the intestines of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

**Table 3-2 Levels of Significant Exposure to RDX - Dermal**

| Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL | LOAEL Less Serious | LOAEL Serious | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|
| **ACUTE EXPOSURE** | | | | | | | |
| **Systemic** | | | | | | | |
| Gn Pig (NS) | once or 3 times | Dermal | 510 mg/kg/day | 1000 mg/kg/day (erythema) | | U.S. Army 1974 | RDX in DMSO |
| Dog (NS) | once | Resp | 289 mg/kg/day | | | U.S. Army 1974 | RDX in DMSO |
| | | Cardio | 289 mg/kg/day | | | | |
| Rabbit (NS) | once | Hemato | 165 mg/kg | | | U.S. Army 1974 | RDX in DMSO (165 mg/kg), RDX in cyclohexanone (37 mg/kg) |
| | | Dermal | | 27 mg/kg (dermatitis) | | | |
| **Neurological** | | | | | | | |
| Dog (NS) | 3 d 1x/day | Cardio | 480 mg/kg/day | | | U.S. Army 1974 | RDX in DMSO |
| **INTERMEDIATE EXPOSURE** | | | | | | | |
| **Systemic** | | | | | | | |
| Gn Pig (NS) | 3 wk 3 d/wk | Dermal | 165 mg | | | U.S. Army 1974 | RDX in DMSO |
| | | Ocular | 165 mg | | | | |

**Table 3-2 Levels of Significant Exposure to RDX - Dermal (continued)**

| Species (Strain) | Exposure/ Duration/ Frequency (Route) | System | NOAEL | LOAEL Less Serious | LOAEL Serious | Reference Chemical Form | Comments |
|---|---|---|---|---|---|---|---|
| Dog (NS) | 4 wk 5 d/wk | Resp | 289 mg/kg/day | | | U.S. Army 1974 | RDX in DMSO |
| | | Cardio | 289 mg/kg/day | | | | |
| | | Ocular | 289 mg/kg/day | | | | |
| Rabbit (NS) | 4 wk 5 d/wk | Resp | 165 mg/kg/day | | | U.S. Army 1974 | RDX in DMSO (165 mg/kg/day), RDX in cyclohexanone (37.5 mg/kg/day) |
| | | Cardio | 165 mg/kg/day | | | | |
| | | Gastro | 165 mg/kg/day | | | | |
| | | Musc/skel | 165 mg/kg/day | | | | |
| | | Hepatic | 165 mg/kg/day | | | | |
| | | Renal | 165 mg/kg/day | | | | |
| | | Dermal | 37.5 mg/kg/day | 165 mg/kg/day (dermatitis) | | | |

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Gn Pig = guinea pig; LOAEL = lowest-observed-adverse-effect level; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

**Hematological Effects.** Blood samples taken from rabbits after a single exposure to 165 mg/kg RDX in DMSO revealed no significant changes in hematological parameters (U.S. Army 1974).

**Musculoskeletal Effects.** Necropsy did not reveal pathology in the muscle or bone tissue of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

**Hepatic Effects.** No alterations in serum clinical chemistry parameters were found in rabbits after acute or intermediate dermal exposure to RDX. Also, no pathological alterations were noted in the liver of rabbits exposed for 4 weeks (U.S. Army 1974).

**Renal Effects.** No histological alterations were noted in the kidneys of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

**Dermal Effects.** One volunteer had a patch of skin covered with dry RDX for 2 days. No irritation was observed following removal of the gauze coverings (von Oettingen et al. 1949). An accurate dose could not be determined because of the lack of information provided in the study. Another study reported dermatitis in workers exposed to RDX fumes of unknown levels and for unknown duration (Sunderman 1944).

Dermatitis was observed in rabbits exposed once to 27 mg/kg RDX in acetone, 37.5 mg/kg RDX in cyclohexanone, or 165 mg/kg RDX in DMSO (U.S. Army 1974); the dermatitis persisted for at least 30 days and was most pronounced in the rabbits exposed to 165 mg/kg RDX in DMSO. Slight erythema was noted in guinea pigs exposed once to 1,000 mg/kg (U.S. Army 1974). Guinea pigs exposed once to an unspecified amount of RDX had exudative dermatitis with edema (Sunderman 1944). The lesions healed promptly after the guinea pigs were removed from the source of exposure.

In rabbits repeatedly exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks, dermatitis was observed after 14 and 30 days of exposure; no dermal effects were observed at 16.5 mg/kg RDX in DMSO or in rabbits administered lower RDX doses in cyclohexanone (37.5 mg/kg/day) or acetone (27 mg/kg/day) vehicles (U.S. Army 1974).

**Ocular Effects.** There are limited human data regarding the ocular toxicity of RDX. Conjunctivitis was reported by workers exposed to RDX fumes (Sunderman 1944); no information was provided regarding exposure levels or duration of exposure.

Cataracts were observed in guinea pigs exposed through cutaneous or intradermal applications of RDX in solvents. However, the incidence of cataracts did not appear to be greater than that found after exposure to the solvents alone. This suggests that RDX itself did not contribute to cataract formation (U.S. Army 1974).

**Body Weight Effects.** Decreased body weight classified as small and transient (no further details were provided) was reported in rabbits after a single dermal application of 2,000 mg/kg of RDX. However, by the end of the observation period, most of the surviving animals showed weight gain (U.S. Army 1984b).

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

#### 3.2.3.3 Immunological and Lymphoreticular Effects

#### 3.2.3.4 Neurological Effects

#### 3.2.3.5 Reproductive Effects

#### 3.2.3.6 Developmental Effects

#### 3.2.3.7 Cancer

## 3.3 GENOTOXICITY

No studies were located regarding genotoxicity of RDX in humans following inhalation, oral, or dermal exposure to the chemical. One *in vitro* study was located in which human fibroblasts (WI-38 cells) were incubated in the presence of RDX and tritiated thymidine (3H-TdR) to measure unscheduled deoxyribonucleic acid (DNA) synthesis (U.S. Army 1978b) (see Table 3-3). RDX was tested in concentrations of up to 4,000 µg/mL both with and without metabolic activation. RDX was not found to significantly increase the rate of unscheduled DNA synthesis in the cells of any exposure group regardless of whether or not metabolic activators were present. Therefore, RDX was not observed to induce DNA damage in human fibroblasts under the conditions of the study (U.S. Army 1978b).

## Table 3-3. Genotoxicity of RDX *In Vitro*

| Species (test system) | End point | Results: With activation | Results: Without activation | Reference |
|---|---|---|---|---|
| Prokaryotic organisms: | | | | |
| *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | Gene mutation | − | − | U.S. Army 1980b |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | Gene mutation | − | − | U.S. Army 1977b |
| *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | Gene mutation | − | − | Whong et al. 1980 |
| *S. typhimurium* TA98, TA100 | Gene mutation | − | − | Lachance et al. 1999 |
| *S. typhimurium* TA98, TA100 | Gene mutation | − | − | George et al. 2001 |
| *S. typhimurium* TA98, TA100 | Gene mutation | − | − | Pan et al. 2007 |
| *S. typhimurium* TA97a | Gene mutation | ± | − | Pan et al. 2007 |
| *Vibrio fischeri* | Gene mutation | ± | ± | Arfsten et al. 1994 |
| Eukaryotic organisms: | | | | |
| Fungi: | | | | |
| *Saccharomyces cerevisiae* | Gene mutation | − | − | U.S. Army 1977b |
| Mammalian cells: | | | | |
| Human fibroblasts | DNA damage | − | − | U.S. Army 1978b |
| Chinese hamster V79 lung cells | Gene mutation | − | − | Lachance et al. 1999 |
| Mouse lymphoma L5178Y cells | Gene mutation | − | − | Reddy et al. 2005a |

— = negative result; + = positive result; ± = weak or equivocal result; DNA = deoxyribonucleic acid

Only two *in vivo* animal studies were located and both provided negative evidence of mutagenicity. U.S. Army (1980b) investigated the effects of oral doses of RDX on dominant lethal mutations in rats. RDX was administered to the rats in the diet in doses of 0, 5, 16, or 50 mg/kg/day for 15 weeks. The males in each exposure group were then allowed to mate with untreated females for 2 weeks. There were no significant effects on the number of corpora lutea, implants, or live or dead embryos (U.S. Army 1980b); no dominant lethal mutations were observed. In the other *in vivo* study, administration of a single gavage dose of up to 250 mg RDX/kg to male mice did not significantly increase the incidence of micronuclei in bone marrow cells examined 24 hours after dosing (Reddy et al. 2005a).

The *in vitro* genotoxicity of RDX has been investigated in several assays (Table 3-3). Most of the results of reverse mutation assays with *Salmonella typhimurium* conducted by several investigators (George et al. 2001; Lachance et al. 1999; Pan et al. 2007; U.S. Army 1977b, 1980b; Whong et al. 1980), *Saccharomyces cerevisiae* (U.S. Army 1977b), or *Vibrio fischeri* (Arfsten et al. 1994) have been negative. A weakly positive result was found in one *S. typhimurium* strain (TA97a) (Pan et al. 2007). In mammalian cells, forward mutation assays in mouse lymphoma L5178Y cells (Reddy et al. 2005a) and hamster V79 lung cells (Lachance et al. 1999) were negative.

Although the results of *in vitro* assays have been negative for RDX, some studies of environmental biotransformation products of RDX have reported positive results. For example, George et al. (2001) reported that hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3-dinitroso-5-nitro-1,3,5,-triazine, were not mutagenic in *S. typhimurium* TA98 or TA100 with or without metabolic activation, but hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) was weakly genotoxic in strain TA100 but negative in strain TA98. Studies conducted by Pan et al. (2007) showed that in the presence of metabolic activation, both MNX and TNX were mutagenic in *S. typhimurium* TA97a, weakly mutagenic in strain TA102, and not mutagenic in strain TA98; in addition, TNX was weakly mutagenic in strain TA100 in the presence of metabolic activation. Pan et al. (2007) also reported that neither MNX nor TNX were mutagenic in *S. typhimurium* TA97a in the absence of metabolic activation.

Collectively, the available information suggests that RDX is not a mutagenic substance, but some of its environmental biotransformation products may be of concern, especially since they have been identified as metabolic products in mammals (Major and Reddy 2007).

## 3.4 TOXICOKINETICS

Very little is known regarding the toxicokinetics of RDX in humans, but reports of adverse effects following inhalation and oral exposure and measurements of RDX in blood from poisoned individuals indicate that RDX is absorbed through the lungs and the gastrointestinal tract. No information is available regarding the distribution and metabolism of RDX in humans. A single case study found RDX in the cerebrospinal fluid following oral exposure (Woody et al. 1986), suggesting possible distribution to the nervous system. RDX was almost completely absorbed in miniature pigs after a single oral dose (Major and Reddy 2007). In rats, mixing RDX with soil considerably reduced absorption compared to administration of neat RDX (Crouse et al. 2008). No preferential accumulation of RDX in specific tissues has been reported in animal studies (Schneider et al. 1977, 1978). Several metabolites were identified in the urine from miniature pigs dosed orally with RDX (Major and Reddy 2007). The urine was the main route of elimination of $^{14}$C-RDX-derived radioactivity (Schneider et al. 1977).

### 3.4.1 Absorption

#### 3.4.1.1 Inhalation Exposure

Neurological effects have been observed in humans following inhalation exposure, indicating that RDX can be absorbed through the lungs (Kaplan et al. 1965; Testud et al. 1996a). The extent of absorption through the lungs has not been determined. As described in an abstract, approximately 30% of an intratracheal dose was excreted in the urine and feces during a 6-day period (Reddy et al. 1989).

#### 3.4.1.2 Oral Exposure

Adverse effects observed in humans following accidental or intentional ingestion of RDX indicate that it is absorbed through the gastrointestinal tract (Davies et al. 2007; Hollander and Colbach 1969; Harrell-Bruder and Hutchins 1995; Kaplan et al. 1965; Kasuske et al. 2009; Ketel and Hughes 1972; Merrill 1968; Stone et al. 1969). Measurements of RDX in blood provide direct evidence that gastrointestinal absorption occurs. A study of a child who ingested an unknown amount of RDX reported an apparent peak plasma concentration prior to 24 hours postingestion (Woody et al. 1986). At 24 hours (first time measurements were made), the serum concentration of RDX was 10.7 μg/mL and decreased gradually thereafter, but it was detectable in serum over a 120-hour period following the estimated time of ingestion. Using an estimated volume of distribution of 2.2 L/kg, Woody et al. (1986) estimated an ingested dose of 84.8 mg RDX/kg or 1.23 g for the 14.5 kg child. More recently, Küçükardalĭ et al.

(2003) reported five cases of accidental ingestion of RDX with estimated doses between 37 and 250 mg/kg (how the doses were estimated was not indicated). Three hours after ingestion, the serum concentrations of RDX ranged from 268 to 969 pg/mL. In two of the patients, blood levels 72 hours after ingestion were approximately 2-fold those measured 3 hours after ingestion, suggesting very slow absorption.

Administration of a single oral dose of $^{14}$C-RDX in 0.5% carboxymethylcellulose in water to miniature pigs resulted in relatively low levels of radioactivity (6% of the dose in males and 2% in females) in the gastrointestinal contents and feces over a 24-hour period, suggesting nearly complete absorption (Major and Reddy 2007). Data shown for a single male pig (two males and two females were used in the study) indicate that Peak RDX concentration in plasma in miniature pigs administered single doses of RDX occurred 8–12 hours after dosing, indicating that although absorption may have been complete over a 24-hour period, it occurred at a relatively slow rate (JHU/U.S. Army 2006; Major and Reddy 2007). In contrast, a study in rats administered 3 or 18 mg/kg RDX via capsule reported Peak RDX levels in the blood 3.5 hours after exposure (Bannon et al. 2009a).

Crouse et al. (2008) studied the bioavailability of RDX from soil in rats. Rats were administered capsules containing neat RDX or RDX mixed with two types of soils. The results showed that administration of RDX mixed with soils resulted in peak blood levels of RDX 15–25% lower that when administered neat. However, the times to reach peak levels (4–6 hours postdosing) did not appear to differ significantly between neat doses of RDX and RDX mixed with soil.

#### 3.4.1.3 Dermal Exposure

A study using excised human skin in flow-through diffusion cells showed poor absorption of RDX in this type of preparation (Reddy et al. 2008). $^{14}$C-RDX was applied in acetone or in two soils differing in their carbon content (1.9 vs. 9.5%) to the epidermal surface and receptor fluid was collected for up to 24 hours. At this time, the RDX remaining on the skin was washed with soap and water and the radioactivity in the washing was counted. Dermal absorption was defined as the amount of radioactivity in the receptor fluid, the dermis, and the portion of the epidermis beneath the stratum corneum. A total of 2.5% of the dose applied in acetone diffused through the skin into the receptor fluid, whereas 5.7% of the applied dose was found in the combined receptor fluid and skin (stratum corneum, epidermis and dermis). Approximately

80% of the applied dose was recovered (receptor fluid plus skin plus washings). Application of RDX in soil resulted in even less absorption; 2.6% in the low-carbon soil and 1.4% in the high-carbon soil were recovered in the receptor fluid and skin in 24 hours.

A similar study using excised pig skin was conducted earlier by Reifenrath et al. (2002). The results also showed relatively poor absorption. Only 4% of the applied dose of RDX in acetone was absorbed over a 24-hour period. Application of RDX mixed with soil resulted in only 1–2% of the applied dose being absorbed.

### 3.4.2 Distribution

#### 3.4.2.1 Inhalation Exposure

No studies were located in humans or animals regarding distribution following inhalation exposure.

#### 3.4.2.2 Oral Exposure

Limited information is available from a study of child who ingested an unknown amount of RDX (Woody et al. 1986). RDX was found in the cerebrospinal fluid of the child at a concentration of 8.94 μg/mL 24 hours after ingestion (only time measured).

In rats given RDX by gavage, levels in the plasma and brain reached a steady state for 2–24 hours and then disappeared 3 days postexposure, but no other tissues were sampled (U.S. Army 1985b). In another single exposure study in rats (Bannon et al. 2009a), blood and brain RDX levels paralleled each other during the first 48 hours post-exposure; these data suggest that RDX did not accumulate in the brain. Miniature swine showed no preferential distribution of RDX to the brain, heart, liver, kidneys, or fat 24 hours following a single gavage dose of 100 mg RDX/kg (Schneider et al. 1977). Three hours after oral administration of RDX to juvenile miniature pigs, the highest levels of RDX were found in the hippocampus and cortex compared to the heart, kidney, liver, blood, lung, and muscle (JHU/U.S. Army 2006). Rats given RDX once by gavage showed the highest levels of RDX in the kidneys, with less in the brain and heart, and the least amount in the plasma and liver over a 24-hour observation period (Schneider et al. 1977). Tissue/plasma ratios during the first 24 hours varied between 0.15 and 10.46, indicating that RDX accumulated to some extent in the tissues examined. In mice administered radiolabelled RDX via stomach perfusion, the highest levels of radioactivity were found in the liver, followed by the kidney, muscle, lung, spleen, heart, and brain (Guo et al. 1985). Results from longer-term studies showed no

preferential distribution of RDX in rats given the chemical by gavage or in the drinking water for 90 days (Schneider et al. 1978). In a recent study of the effects RDX on gene expression in the brain of rats, administration of 3 or 18 mg RDX/kg in a capsule resulted in peak brain and blood concentrations of RDX approximately 3.5 hours after dosing, regardless of the dose (Bannon et al. 2009a). No RDX could be detected in the brain or blood from low-dose rats 24 hours after dosing or in blood or brain from high-dose rats 48 hours after dosing.

An unpublished study indicates that RDX was found in the brain of rat pups whose mothers were administered RDX from gestation day 6 through postnatal day 10 (U.S. Army 2007b). On postnatal days 0, 3, 5, and 10, dams and pups were tested for RDX in milk and brain, respectively. Significantly higher concentrations of RDX were found in the brain from pups sacrificed immediately after birth than in the brain of pups sacrificed on postnatal day 10. No explanation was offered for this finding by the investigators. It is plausible that the gastrointestinal tract of newborn pups did not absorb RDX, but RDX readily crossed the placenta. Alternatively, it could be that newborn pups have the ability to metabolize/excrete RDX that is not present in the fetus. In any case, transplacental exposure occurred. Since RDX was also found in the dam's milk, transfer of RDX to the offspring via the milk can also occur.

#### 3.4.2.3 Dermal Exposure

No studies were located in humans or animals regarding distribution following dermal exposure.

### 3.4.3 Metabolism

There are no studies available regarding RDX metabolism in humans following inhalation, oral, or dermal exposure.

RDX was extensively metabolized in rats (Schneider et al. 1977). Administration of a single gavage dose of 50 mg $^{14}$C-RDX/kg resulted in <0.6% of the dose in the carcass 4 days after dosing and only 3% was excreted unchanged, mostly in the urine. The metabolites were not characterized.

A study of the metabolism of RDX in miniature pigs showed that RDX is rapidly and extensively metabolized by loss of two nitro groups followed by ring cleavage (Major and Reddy 2007). Pigs were administered a single gavage dose (43 mg/kg) of $^{14}$C-RDX combined with carboxymethylcellulose in water and blood and excreta were collected for up to 24 hours. Metabolites were characterized by liquid

chromatography/mass spectrometry (LC/MS) in selected samples of urine, plasma, and liver. Analysis of urine revealed two major metabolites, 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide. Using a more sensitive method of analysis, the investigators also identified MNX in both male and female urine and DNX in male urine. Analysis of plasma showed quantifiable amounts of RDX, and trace levels of MNX, DNX, and TNX. Analysis of liver extracts showed that most of the radioactivity was in the form of water-soluble, high-molecular-weight compounds rather than as RDX or any identifiable metabolites.

An *in vitro* study examining RDX metabolism (assessed by measuring loss of RDX) under low oxygen conditions determined that 46.6, 40.1, 34.6, 25.5, and 11.6% of the RDX was metabolized in human, rat, monkey, pig, and rabbit liver microsomes, respectively, following a 30-minute incubation period (U.S. Army 2008). After a 180-minute incubation period, 51.8, 47.2, 35.7, 33.7, and 18.0% of the RDX was metabolized, respectively. Under anaerobic conditions with nitrogen replacing oxygen, RDX was metabolized by several human recombinant cytochrome P450 isoforms (CYP1A1, CYP2B6, CYP2C8, CYP2C18, CYP2E1, CYP3A5); with the exception of CYP1A1, the RDX metabolite, MEDINA, was produced. In contrast, under aerobic conditions, no loss of RDX was detected in human liver microsomes, S9, hepatocytes, or a number of human recombinant cytochrome 450 isoforms (U.S. Army 2008).

RDX was metabolized *in vitro* by rabbit cytochrome CYP2B4 to 4-nitro-2,4-diazabutanal, nitrite, formaldehyde, and ammonia (Bhushan et al. 2003). This reaction was observed in a cell-free, isolated enzyme system; therefore, it's relevance to *in vivo* metabolism is unknown.

### 3.4.4 Elimination and Excretion

#### 3.4.4.1 Inhalation Exposure

No relevant information was located from studies in humans. In rats receiving a single intratracheal dose of radiolabelled RDX, 23 and 3% of the label was excreted in the urine and feces, respectively, during the first 4 days; during the first 6 days, 26 and 5%, respectively, was excreted (Reddy et al. 1989; only available as an abstract).

#### 3.4.4.2 Oral Exposure

Only one study is available that provides some data on excretion in humans after oral exposure. In a child who ingested an unknown amount of RDX, apparent peak concentration in urine occurred at

approximately 48 hours after ingestion and in feces 96 hours after ingestion (Woody et al. 1986). RDX could still be detected in feces 144 hours following ingestion.

Rats given a single radiolabeled gavage dose of RDX eliminated 43% of the radioactivity in exhaled air, 34% in the urine, and 3% in the feces within 4 days; about 10% remained in the carcass (Schneider et al. 1977). A longer-term study showed similar excretion patterns; during a continuous drinking water study, 50% was eliminated in the exhaled air, 34% in the urine, and 5% in the feces (Schneider et al. 1978). There was no evidence that RDX accumulated in the tissues during longer-term exposure. Following administration of radiolabelled RDX to mice via stomach perfusion, 38.18% of the dose was excreted in the urine and 26.64% was excreted in the feces on day 1. On days 2–9, 11.20% of the dose was excreted in the urine. Ten days after dosing, 75.25% of the dose was excreted in the urine and feces (Guo et al. 1985).

Urine was the major route of elimination of $^{14}$C-RDX-derived radioactivity in miniature pigs given a single dose of the chemical (Major and Reddy 2007). Over a 24-hour period, 16–17% of the administered dose was recovered in the urine compared to ≤6% recovered in the gastrointestinal contents and feces.

#### 3.4.4.3 Dermal Exposure

No relevant information was located from studies in humans or animals.

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

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen 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.

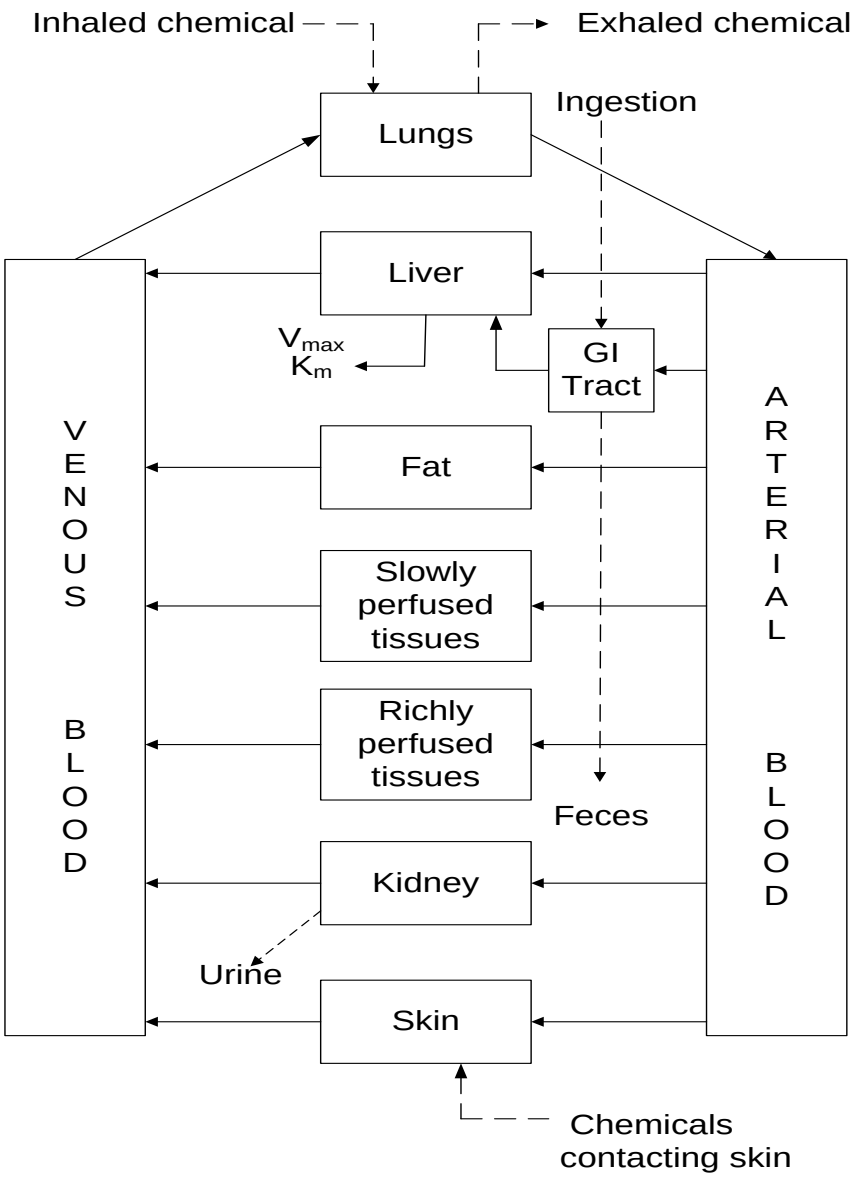
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically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

If PBPK models for RDX 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.

**Figure 3-2. 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

### Krishnan Model (Krishnan et al. 2009; U.S. Army 2007a; Sweeney et al. 2012)

**Description of the Model.** Krishnan et al. (2009; U.S. Army 2007a) developed a PBPK model for simulating kinetics of RDX in rats. The model was subsequently modified and extended to include simulations of human kinetics (Sweeney et al. 2012). The structure of the model is essentially identical to the generic model depicted in Figure 3-2, with the following tissue compartments: brain, fat, liver, richly perfused tissues (RPT), and slowly perfused tissues (SPT). Parameters and parameter values reported in Sweeney et al. (2012) are presented in Table 3-4.

The model simulates absorption of RDX from the gastrointestinal tract as first order transfers to liver from stomach (KAS, $hour^{-1}$) and duodenum (KAD, $hour^{-1}$), with first-order transfer from stomach contents to duodenum contents (KT, $hour^{-1}$). Since no other transfers from the gastrointestinal tract are simulated (i.e., transfer to lower gastrointestinal-tract or fecal excretion), 100% of the oral dose is eventually absorbed at infinite time after an oral dose. Distribution of RDX to tissues is simulated as flow-limited transfers in which instantaneous partitioning of RDX between tissue and blood is assumed, the tissue-venous blood concentration ratio is given by a tissue-blood partition coefficient, and blood-tissue clearance (L/hour) is assumed to be equivalent to tissue blood flow. Elimination of absorbed RDX is assumed to be entirely by metabolism, all of which is attributed to the liver, and is simulated as a first order processes (KfC, $kg^{0.33}/hour^{-1}$). Metabolites of RDX are not simulated in the model.

Sweeney et al. (2012) derived values for several parameters in the rat model, based on statistical optimization of predicted blood concentration kinetics against observations from rat studies not used for parameter estimation by Krishnan et al. (2009). A value for a single absorption rate constant in rats was estimated by Krishnan et al. (2009) based on model performance (visual inspection of fit to observations) in simulating blood RDX kinetics in rats that received a single oral dose of RDX (Schneider et al. 1977). Sweeney et al. (2012) derived alternative values for a two-compartment gastrointestinal model (stomach, duodenum) by optimization against observed blood RDX kinetics from various rat oral studies (Bannon et al. 2009a; Crouse et al. 2008; Krishnan et al. 2009).

Tissue:blood partition coefficients for RDX were estimated by Krishnan et al. (2009) based on a measured n-octanol:water partition coefficient for RDX, and reported water and lipid contents of specific tissues. The value for the metabolism rate constant was estimated by evaluating alternative values against

## Table 3-4. Parameter Values for Sweeney et al.(2012) PBPK Model of RDX in Rats and Humans

| Description | Value | | Source |
|---|---|---|---|
| | Rat | Human | |
| Body weight (*BW*, kg) | 0.3 | 70 | Observed |
| Cardiac output (*KQC*, L/hour/kg$^{0.74}$) | 15 | 14 | Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002 |
| Blood flow (K*Q*) fraction of cardiac output | | | |
| Liver (K*QL*) | 0.25 | 0.175 | Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002 |
| Brain (*KQB*) | 0.03 | 0.114 | |
| Fat (*KQF)* | 0.09 | 0.085 | |
| Slowly perfused tissues (*KQS*) | 0.20 | 0.2449 | |
| Rapidly perfused tissues (*KQR*) | 0.43 | 0.3811 | 1-(*KQL+KQB+KQF+KQS*) |
| Compartment volumes ($V_i$) fraction of body weight | | | |
| Liver (*KVL)* | 0.04 | 0.026 | Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002 |
| Brain (*KVB*) | 0.012 | 0.02 | |
| Fat (*KVF)* | 0.07 | 0.21 | |
| Rapidly perfused tissues (*KVR*) | 0.04 | 0.052 | |
| Blood (*KVV*) | 0.06 | 0.079 | |
| Slowly perfused tissues (*KVS*) | 0.688 | 0.523 | 0.91 – (*KVL+KVB+KVF+KVR+KVV*) |
| Tissue:blood partition coefficients | | | |
| Liver *(PL*) | 1.2 | 1.3 | Krishnan et al. 2009 (predicted from n-octanol:water partition coefficient) |
| Brain (*PB*) | 1.4 | 1.6 | |
| Rapidly perfused tissues (*PS*) | 1.4 | 1.6 | |
| Fat (*PF*) | 5.57 | 5.57 | Optimized—intravenous rat data[a] |
| Slowly perfused tissues (*PR*) | 0.15 | 0.15 | |
| Liver metabolism | | | |
| Metabolism (*KfC*, kg$^{0.33}$/hour) | 2.6 | 11.2 | Optimized—intravenous rat data[a] Optimized—oral human data[b] |
| Gastrointestinal absorption | | | |
| Absorption from stomach (*KAS*, hour$^{-1}$) | | 0.033 | Optimized—oral human data[b] |
| gavage (rat) | 0.83 | NA | Optimized—oral rat data[c] |
| capsule (rat) | 0.12 | NA | |
| coarse (rat) | 0.005 | NA | |
| Transfer to duodenum (*KT*, hour$^{-1}$) | | 0 | Optimized—oral human data[b] |
| gavage (rat) | 1.37 | NA | Optimized—oral rat data[c] |
| capsule (rat) | 0 | NA | |
| coarse (rat) | 0 | NA | |
| Absorption from duodenum (*KAD*, hour$^{-1}$) | | NA | Optimized—oral human data[b] |
| gavage (rat) | 0.0258 | NA | Optimized—oral rat data[a] |
| capsule (rat) | NA | NA | |
| coarse (rat) | NA | NA | |

[a]Krishnan et al. 2009.
[b]Özhan et al. 2003; Woody et al. 1986.
[c]Bannon et al. 2009a; Crouse et al. 2008; Krishnan et al. 2009; Schneider et al. 1977.

Source: adapted from Sweeney et al. 2012

observed blood RDX kinetics following intravenous dosing of rats with RDX (Schneider et al. 1977). Sweeney et al. (2012) re-evaluated the values for the partition coefficients for adipose and slowly perfused tissue, and the metabolism rate constant, and simultaneously optimized all three parameters against blood RDX kinetics from a rat intravenous study conducted by Krishnan et al. (2009). The simultaneous optimization yielded values of 5.57 and 0.15 for the partition coefficients for adipose and slowly perfused tissue, respectively, whereas Krishnan et al. (2009) estimated values of 7.55 and 1. The optimized value for the metabolism rate constant was 2.6 $kg^{0.3}$/hour, whereas Krishnan et al. (2009) estimated the value to be 2.6 kg/hour. For a 0.4 kg rat (Krishnan et al. 2009), the corresponding values for the metabolism rates constants are 3.4 $hours^{-1}$, based on the Sweeney et al. (2012) estimate, and 5.5 $hours^{-1}$, based on the Krishnan et al. (2009) estimate.

Parameter values for the human model were scaled to body weight (e.g., flows scaled to $BW^{0.74}$ and volumes to $BW^{1}$), with the exception of the metabolism and absorption rate constants. Absorption and metabolism rate constants for humans were optimized against observations of blood RDX kinetics in cases of ingestion exposures in humans (Özhan et al. 2003; Woody et al. 1986). Because doses in the human cases were unknown, dose was also optimized for each case. The estimated parameter values based on simulation of plasma RDX concentrations in a 3-year old child who ingested an unknown amount of RDX (Woody et al. 1986) were: dose 58.9 mg/kg; KAS 0.060/hour; KfC 9.87 $kg^{0.33}$/hour. Corresponding values based on plasma RDX kinetics in adults were: dose 3.5 mg/kg; KAS 0.033/hour; KfC 11.2 $kg^{0.33}$/hour. Sweeney et al. (2012) also estimated the value for the first-order metabolism rate constant based on scaling of the rat value against the ratio of *in vitro* metabolism rates in rats and humans, adjusted for microsomal protein (Lipscomb and Poet 2008, as cited in Sweeney et al. 2012; U.S. Army 2008) as follows:

$$KfC_h = KfC_r \cdot MRR_{h/r} \cdot MSPR_{h/r} \cdot BWR_{h:r}^{0.33}$$

where KfC is the first-order metabolism rate constant in human or rat (human or rat, respectively), MRR is the *in vitro* metabolism rate ratio (human:rat), MSP is the microsomal protein yield ratio (human:rat), and BWR is the body weight ratio (human:rat). The estimated values for the metabolism rate constant in humans was 12.4 $kg^{0.33}$/hour, which was similar to the value for adults estimated by optimization against the Özhan et al. (2003) data.

**Validation of the Model.** Krishnan et al. (2009) estimated the metabolism rate constant based on blood RDX kinetics obtained from an intravenous study in rats (Schneider et al. 1977), and then evaluated model performance against data obtained from a different intravenous study conducted in rats (Krishnan et al. 2009). The same approach was used to evaluate the gastrointestinal absorption rate constant; data from a study in which rats received a single gavage dose of RDX (Krishnan et al. 2009) were used to estimate the parameter values, and the resulting model was evaluated against data from a different gavage rat study (Schneider et al. 1977). In both evaluations, the studies that were used to evaluate the model administered lower doses than the studies used to estimate parameter values.

As previously described, Sweeney et al. (2012) optimized the absorption and metabolism parameter values, and tissue:blood partition coefficients for adipose and SPT in the rat using data from intravenous and oral dosing studies reported in Krishnan et al. (2009). The results of optimization of the intravenous studies were compared to an independent data set (Schneider et al. 1977) and the results were summarized with the following conclusion: *"…the agreement between the model and the iv data of Schneier eta l. (1977) was very good (not shown)."* In the oral dosing studies, rats received a gavage dose of RDX dissolved in water. The absorption parameters were re-optimized to simulate blood RDX kinetics in studies in which RDX was administered as a granular RDX (coarse) or in a gelatin capsule, or as a suspension in water (Bannon et al. 2009a; Crouse et al. 2008; Schneider et al. 1977). One of the studies included measurements of brain RDX concentrations (Bannon et al. 2009a). Since these studies were used to calibrate the absorption kinetics parameters to account for the different dosing formulations, they do not represent a fully independent validation of the rat model for simulating oral dosing. However, these evaluations do allow validation of the simulations of distribution and elimination kinetics derived from intravenous studies. Blood RDX kinetics obtained for cases of human ingestion of RDX were used to estimate values for absorption and metabolism parameters in the human. The human model was not evaluated against observations independent of those used to estimate parameter values.

All of the model calibration and evaluation studies were conducted with data from single-dose studies. Although Sweeney et al. (2012) used a statistical procedure (maximum likelihood) to estimate parameter values, statistical comparisons of goodness of fit were not reported in Sweeney et al. (2012), and were not reported in Krishnan et al. (2009).

**Risk Assessment.** The RDX model predicts blood and brain levels of RDX that would occur in association with oral doses to RDX. These predictions are potentially useful for predicting internal doses of RDX in rats and/or humans (e.g., blood or brain concentrations), and for making extrapolations of

these internal dose metrics across species. The model has been used to extrapolate dosages in repeated oral dosing studies in rats to equivalent oral dosages in humans in a derivation of human equivalent external doses and candidate chronic oral reference doses (Sweeney et al. 2012). Several internal dose metrics were explored in the interspecies dosimetry extrapolation. Peak and average brain RDX concentrations were used for dosimetry of neurological end points observed in rats in intermediate gavage and chronic dietary studies (U.S. Army 1983a, 2006). The time-weighted average RDX concentration in richly perfused tissue and steady-state body weight-adjusted rate of metabolism were used for dosimetry of prostate inflammation observed in rats in a chronic dietary study (U.S. Army 1984c) and alterations in survival time, terminal body weight, and hematocrit and hemoglobin concentrations in rats in a chronic dietary study (U.S. Army 1984c). Sweeney et al. (2012) estimated human equivalent external doses ranging from 1.6 to 8 mg/kg/day.

**Target Tissues.** The RDX model was calibrated to predict blood RDX kinetics following oral exposures to RDX, although it also predicts concentrations in brain and other tissues. Sweeney et al. (2012) presented simulations of brain RDX concentration in comparison to measurements made in rats (Bannon et al. 2009a). The model has been used to predict concentrations of RDX in brain, which has been shown to be an important toxicity target tissue for RDX (Sweeney et al. 2012).

**Species Extrapolation.** Sweeney et al. (2012) scaled the rat model to humans using a combination of allometric scaling and optimization of selected model parameters (absorption and metabolism rate constants). The scaled human model has not been evaluated against independent observations not used to estimate model parameter values. The model has been used to extrapolate rat dosages to humans based on predicted internal dosimetry (Sweeney et al. 2012).

**Interroute Extrapolation.** The RDX model as it is currently configured simulates RDX kinetics associated with intravenous and oral dosing. Simulation of other potential routes of exposure (e.g., inhalation, dermal) would require development of models for the absorption of inhaled RDX, or RDX deposited on the skin.

**Strengths and Limitations.** Strengths of the model are that it simulates disposition and clearance of intravenously injected or ingested RDX in rodents and humans, including predicting levels of RDX in the brain, a target tissue for toxicity. However, limitations include: (1) all model calibration and evaluation studies were conducted with data from single-dose studies and confidence in simulating RDX kinetics of repeated dosing schedules has not been evaluated; (2) the human model was not evaluated against

observations independent of those used to estimate parameter values; and (3) the validity of predictions of brain levels of RDX in humans is based solely on performance of the model in predicting observed blood kinetics in humans who ingested unknown doses of RDX (dose was optimized to the blood RDX data).

## 3.5 MECHANISMS OF ACTION

### 3.5.1 Pharmacokinetic Mechanisms

**Absorption.** The mechanism(s) of absorption of RDX is not known. There are no studies that calculated rates of absorption that could have provided some indication of a possible mechanism of absorption. In rats administered RDX in a capsule, peak blood concentrations were achieved 4–6 hours after dosing (Crouse et al. 2008). In a male miniature pig given a single gavage dose of RDX as a suspension in 0.5% carboxymethylcellulose in water, peak plasma concentration of RDX occurred at approximately 12 hours after dosing, which would suggest a relatively low rate of absorption. Studies with excised human and pig skin showed that mixing RDX with soil significantly reduced dermal absorption relative to RDX neat (Reddy et al. 2008; Reifenrath et al. 2002).

**Distribution.** No specific mechanism of distribution was apparent in the available studies. In rats, the distribution of RDX (single doses) seemed unaffected by the route of administration (parenteral vs. oral) or by the dose (Schneider et al. 1977). The concentration of RDX-derived radioactivity in most tissues was fairly stable between 2 and 24 hours after dosing except in the liver, where it fluctuated widely. High concentrations of radioactivity occurred in the liver at 2, 12, and 24 hours after dosing, which led Schneider et al. (1978) to suggest that there might be diurnal variations in the hepatic metabolism of RDX. In 90-day studies, RDX did not accumulate in any of the tissues examined (Schneider et al. 1978).

**Metabolism.** The metabolism of RDX has been studied in some detail in miniature pigs (Major and Reddy 2007) and there is some evidence suggesting that a cytochrome orthologue to the rabbit, CYP2B4, may be involved (Bhushan et al. 2003). The two major metabolites characterized were 4-nitro-2,4-diaza-butanal and 4-nitro-2,4-diazabutanamide. Trace amount of MNX, DNX, and TNX were also detected. Some studies have provided some information regarding the role of metabolism in the toxicity of RDX. In rats, administration of RDX intravenously resulted in convulsive activity within seconds after the injection, which suggested that the convulsions are produced by the parent compound (Schneider et al. 1977). In a 90-day gavage study in monkeys, convulsive events were associated with higher RDX concentrations in plasma (U.S. Navy 1974b), which would also support the idea of the parent compound being responsible for the convulsive activity. More recently, Meyer et al. (2005) reported that MNX and

RDX were equipotent in inducing convulsions and lethality in female Sprague-Dawley rats in single-dose gavage studies of 14-day duration; both DNX and TNX were less potent. In a study of age-dependent acute toxicity of RDX in deer mice, Smith et al. (2007) reported that, for all three age brackets tested, RDX was significantly more potent than MNX and TNX.

**Excretion.** The urine and exhaled $CO_2$ were the main routes of excretion of $^{14}C$-RDX-derived radioactivity in rats following acute- or intermediate-duration exposure to RDX (Schneider et al. 1977, 1978). In the acute studies, only 3% of the administered radioactivity was recovered in the feces over a 4-day period (Schneider et al. 1977). The urine was also the main excretory route of radioactivity in miniature pigs following a single gavage dose of RDX (Major and Reddy 2007). No information was located regarding how the size of the dose might affect the distribution of metabolic products among excretory pathways.

### 3.5.2 Mechanisms of Toxicity

The main effect of high doses of RDX in humans and animals is the induction of hyperactivity manifested as convulsions or seizures. RDX has also induced other effects; however, because these effects have not been well characterized and/or have been seen inconsistently in animal studies, this section will focus mainly on the potential mechanisms of neurological effects. *In vitro* studies in primary human cells, including neurons, astrocytes, and microglia cells, have found minimal evidence of cytotoxicity (U.S. Army 2010), suggesting that the observed neurological effects of RDX are likely to be reversible effects on neurotransmission. Hyperactivity can result from a chemical acting centrally and/or on the peripheral nervous system. Chemicals such as organophosphorus pesticides or nerve agents, such as sarin and soman, act mainly by inhibiting cholinesterase activity in the brain (McDonough and Shih 1997), but limited information is available regarding possible effects of RDX on cholinesterase activity. Based purely on the chemical structure of RDX, it seems unlikely that it would possess potent anticholinesterase properties. In rats receiving a single intraperitoneal dose of RDX, small, but significant, decreases in brain cholinesterase levels were found 1.5, 3, or 6 hours after dosing. By 24 hours after dosing, the cholinesterase levels were similar to controls (Maryland University 1975). However, in rats receiving 2.5 or 6.5 mg/kg/day RDX administered intraperitoneally for 6 or 12 weeks, significant increases in brain cholinesterase levels were found. An *in vitro* study found a 53% decrease in cholinesterase activity in brain homogenates incubated with $4.5x10^{-3}$ M RDX (Maryland University 1975). In contrast, no alterations in frontal lobe or blood acetylcholinesterase activity were observed at the onset of seizures in rats administered a single gavage dose of 75 mg/kg RDX (Williams et al. 2011). The Maryland

University study (1975) also found significant increases in monoamine oxidase activity in rats receiving intraperitoneal doses of 2.5 or 6.5 mg/kg/day RDX for 6 or 12 weeks or 0.3 mg/kg/day for 12 weeks. However, following a single dose, a small, but not statistically significant, decrease in monoamine oxidase activity was observed 0.5, 1.5, 3, 6, or 24 hours after dosing. As with cholinesterase activity, RDX induced a dose-related decrease in monoamine oxidase activity *in vitro*.

Studies in rats by Burdette et al. (1988) suggested that limbic structures may be a primary target for RDX toxicity. The suggestion was based on observations of spontaneous seizure characteristics and an accelerated rate of amygdaloid kindling following administration of a subconvulsive dose of RDX. Recent studies provide evidence of the involvement of GABA (γ-aminobutyric acid) receptors in RDX-induced neurologic dysfunction. Antagonism of GABAergic neurons within the central nervous system leads to generalized nervous system stimulation. Binding of GABA to its receptor opens chloride-selective ion channels leading to influx of chloride into neurons through an electrochemical gradient resulting in hyperpolarization of the membrane and inhibition of cell firing. A reduced inhibitory drive results in uninhibited activity in effector neurons. Williams et al. (2011) found that RDX binds to the picrotoxin convulsant site on $GABA_A$ receptors, but did not bind to other neurotransmitter receptors that are targets of other known convulsants, including the glutamate family of receptors, nicotinic and muscarinic acetylcholine receptors, the glycine receptors, and the batrachotoxin site of the sodium channel. This finding is supported by 3-D modeling, which found that RDX does not appear to be a ligand for the N-methyl-D-aspartate-glutamate receptor in postsynaptic neurons (Ford-Green et al. 2011). *In vitro*, RDX reduced the frequency and amplitude of spontaneous $GABA_A$ receptor-mediated inhibitory postsynaptic currents and the amplitude of GABA-evoked postsynaptic currents in the rat basolateral amygdala. Williams et al. (2011) also found a significant negative correlation between the levels of RDX in the brain and the time to seizure onset in rats administered 75 mg/kg RDX via gavage. These findings suggest that the convulsions were due to parent compound rather than a metabolite. Similarly, a study of Northern bobwhite quail found 20 times higher brain RDX levels in birds with seizures, compared to birds exposed to the same dosage but did not develop seizures (Gust et al. 2009).

Some support for the hypothesis of an RDX-induced imbalance between inhibitory and excitatory systems is provided by a recent study of global gene expression in the brain of rats dosed with either 3 or 18 mg RDX/kg (Bannon et al. 2009a). Relative to low-dose rats, gene expression in the cerebral cortex of high-dose rats was significantly decreased, particularly for processes related to the generation, packaging, mobilization, and release of neurotransmitters. Significantly down-regulated was the glutamate signaling pathway, which could be a response to excessive excitation resulting from the removal of the inhibition

by GABAergic pathways, caused in turn by RDX. *In vitro* studies using human neuroblastoma cells found an RDX-induced transient increase in calcium levels (released from intracellular calcium stores) (Ehrich et al. 2009); this increase in calcium may mediate the release of glutamate. Genomic results from a study of Northern bobwhite quail found significant alterations in the differential expression of transcripts involved the electrophysiology and signal transduction of neurons (Gust et al. 2009). The investigators suggested that these alterations may result in an inhibition of neuronal cell repolarization postaction potential leading to heightened neuronal excitability and seizures. Zhang and Pan (2009) found significant alterations in the number of microRNAs (miRNAs) expressed and expression levels in the brains of mice exposed to low levels of RDX in the diet for 28 days. The most affected miRNA was MiR-206, which was significantly up-regulated in the brain. The brain-derived neurotrophic factors (BDNF) gene is a potential miRNA target. Zhang and Pan (2009) speculated that miR-206 may contribute to the neurological effects associated with RDX exposure through its reduction of BDNF gene expression. The results of this study should be interpreted cautiously; additional research is needed to evaluate the role of altered miRNA expression in RDX toxicity. Bannon et al. (2009b) noted that the significance of miRNA as a predictor of toxic insult or disease has not been demonstrated.

### 3.5.3 Animal-to-Human Extrapolations

Virtually all of the information regarding the effects of RDX is derived from cases of acute exposure to doses of RDX that induced frank effects. In both humans and animals, high doses of RDX affect primarily the nervous system. However, which experimental animal is the best model for human exposure is unknown, although the basic mechanism for seizure induction is probably the same in humans and animals. Studies in animals have provided enough information to establish approximate blood levels of RDX that are associated with convulsive activity (Burdette et al. 1988; Schneider et al. 1977, 1978; U.S. Navy 1974b). That information is lacking in humans. Only two of the numerous case reports available measured RDX in the blood of the patients, Woody et al. (1986) and Küçükardalĭ et al. (2003). Blood levels of RDX reported by Woody et al. (1986) appear to be consistent with what has been measured in animal studies administered doses similar to those estimated for the patient in Woody et al. (1986). However, blood levels of RDX reported by Küçükardalĭ et al. (2003) were at least 3 orders of magnitude lower, even though the doses that the investigators estimated the patients had consumed (37–250 mg RDX/kg) were in the range of that estimated by Woody et al. (1986) (84.8 mg RDX/kg). No explanation was offered by Küçükardalĭ et al. (2003) for this discrepancy. Although there are limited data on the toxicokinetics of RDX in humans, the Krishnan PBPK model (Krishnan et al. 2009; Sweeney et al. 2012; U.S. Army 2007a) allows for extrapolation of the results of animal studies to humans.

## 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 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 isoflavinoid 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).

There is no evidence suggesting that the reproductive and developmental effects reported in animals summarized in Sections 3.2.2.5 and 3.2.2.6, respectively, involve actions of RDX on the neuroendocrine axis. No *in vitro* studies were located regarding endocrine disruption of RDX.

## 3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient

tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

There are limited data on the toxicity and toxicokinetic properties of RDX in children. Clonic-tonic convulsions were reported in a 3-year-old child ingesting RDX (Woody et al. 1986). As described in the case-report, the observed effects are similar to those observed in adults (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969). The lack of adequate exposure data in most of these cases precludes evaluating whether children are more susceptible to RDX toxicity. Age-specific differences in $LD_{50}$ values were found in deer mice; 21-day-old mice were the most sensitive followed by 200- and 50-day-old animals (Smith et al. 2007). The $LD_{50}$ in the 50-day-old mice was approximately twice as great as the value in 200-day-old mice. It is not known if similar differences would occur for other toxic effects.

As discussed in greater detail in Section 3.2.2.6, developmental effects (decreases in growth and survival) have been observed in the offspring of rats orally exposed to RDX (U.S. Army 1980b, 1986d). These effects were typically observed at doses associated with RDX-induced seizures in the dams and may not have been a direct effect on the fetus/pup. No developmental effects have been observed in rabbits (U.S. Army 1980b).

## 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 RDX 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 RDX are discussed in Section 3.8.2.

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

### 3.8.1 Biomarkers Used to Identify or Quantify Exposure to RDX

RDX was detected in the serum, urine, and feces of a child who consumed unknown levels of RDX in the form of C-4 (91% RDX). RDX was measured in the serum for 120 hours and in the feces for 144 hours after the presumed time of ingestion (Woody et al. 1986). RDX was also measured in plasma from five

male cases described by Küçükardalĭ et al. (2003). Therefore, the chemical itself is a specific biomarker of exposure. Since the metabolism of RDX in humans has not been studied, it is not known whether single measurements of RDX in blood or urine could be used only as a biomarker of recent exposure or also as a biomarker of low-level prolonged exposure.

### 3.8.2 Biomarkers Used to Characterize Effects Caused by RDX

High oral doses of RDX are known to produce seizures in humans (Davies et al. 2007; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) and animals (Burdette et al. 1988; Schneider et al. 1977; U.S. Army 1983a; U.S. Navy 1974b; von Oettingen et al. 1949), but this effect is not specific to RDX. Thus, there are no known specific biomarkers to characterize effects caused by inhalation, oral, or dermal exposure to RDX.

## 3.9 INTERACTIONS WITH OTHER CHEMICALS

Many of the human studies on the accidental inhalation or ingestion of RDX involved composition C-4, which was used for demolition by the U.S. Armed Forces during the Vietnam War. Composition C-4 was 91% RDX, with the other components consisting of polyisobutylene, motor oil, and 2-ethylhexyl sebacate. Minimal information is available on the toxicological properties of these components of C-4, and it is not known whether they may contribute to the effects seen from exposure to C-4. However, since RDX is the primary component of C-4, the assumption has been made that the major effects noted from C-4 are due to RDX. In addition, the human and animal reports of ingested RDX usually are not limited to pure RDX, but are almost always reports of RDX contaminated with octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) or other substances. There are no studies regarding the interactions of these substances. However, there are several studies in which the oral toxicity of trinitrotoluene (TNT) and RDX were investigated. In one study (Levine et al. 1990), TNT and RDX were co-administered in the feed of rats for 13 weeks. This co-administration potentiated the decrease in body weight gain as compared to RDX alone. TNT antagonized the lethal effects and the hypotriglyceridemia induced by RDX. RDX antagonized the hypercholesterolemia, splenomegaly, testicular atrophy, hepatocytomegaly, degeneration of the seminiferous tubules, and pigmentation of renal cortices induced by TNT.

## 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to RDX than will most persons exposed to the same level of RDX 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 RDX, or compromised function of organs affected by RDX. Populations who are at greater risk due to their unusually high exposure to RDX are discussed in Section 6.7, Populations with Potentially High Exposures.

There are no known populations that would be unusually susceptible to RDX toxicity because of their genetic make-up, developmental stage, health status, nutritional status, or chemical exposure history.

## 3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

### 3.11.1 Reducing Peak Absorption Following Exposure

A general recommendation for reducing absorption after inhalation exposure to RDX is to move the patient to fresh air (HSDB 2009). Emesis is not recommended following oral exposure because of the probability of developing seizures (HSDB 2009). Charcoal may be administered to reduce absorption following oral exposure. The only information located for reducing absorption following dermal exposure specifically of RDX is a study by Twibell et al. (1984) which reported that washing the hands immediately after handling RDX can remove approximately 90% of the residue. Information summarized by HSDB (2009) suggests removing contaminated clothing and washing the exposed area thoroughly with soap and water. In case of eye contact, irrigation of the exposed eyes with copious amounts of room temperature water for at least 15 minutes is recommended.

### 3.11.2 Reducing Body Burden

No information was located on specific methods for reducing the body burden of RDX. However, Küçükardalĭ et al. (2003) reported that hemodialysis was unsuccessful in reducing the serum levels of RDX in three cases of oral intoxication with the chemical when performed approximately 3 hours after ingestion.

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

The primary adverse effect of RDX is the induction of convulsive activity and seizures for which standard treatments are available. Intravenous administration of a benzodiazepine such as diazepam or lorazepam is recommended (HSDB 2009). If seizures recur after diazepam, phenobarbital or propofol should be considered (30 mg for adults or 10 mg for children older than 5 years).

## 3.12 ADEQUACY OF THE DATABASE

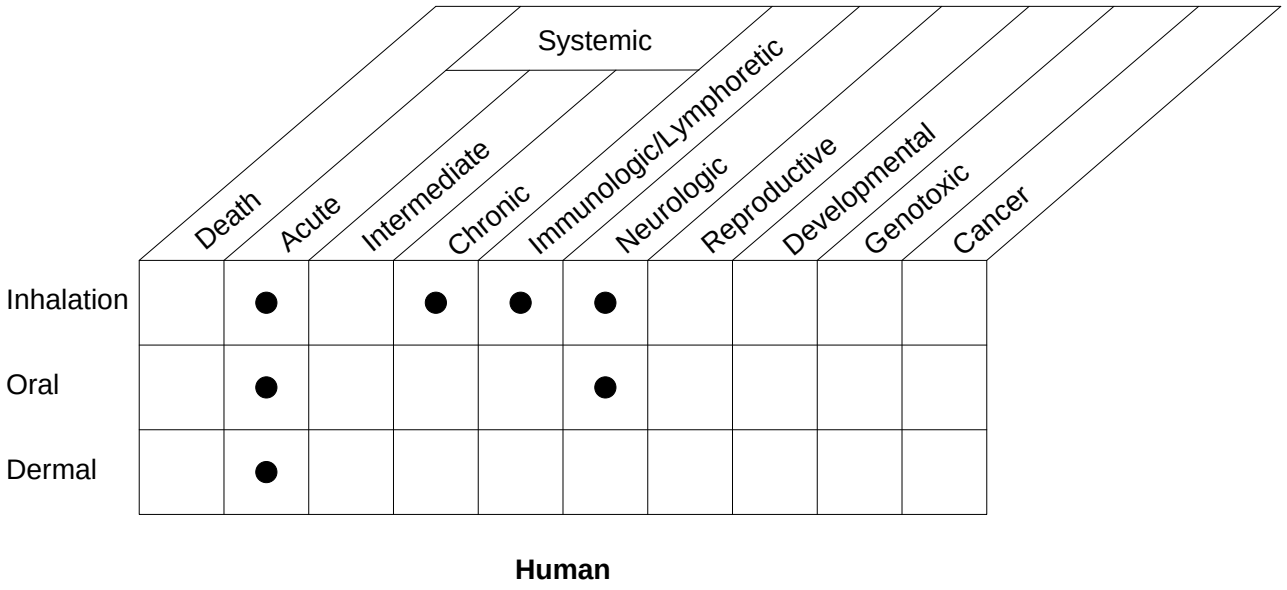
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of RDX is available. Where adequate information is not available, 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 RDX.

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.

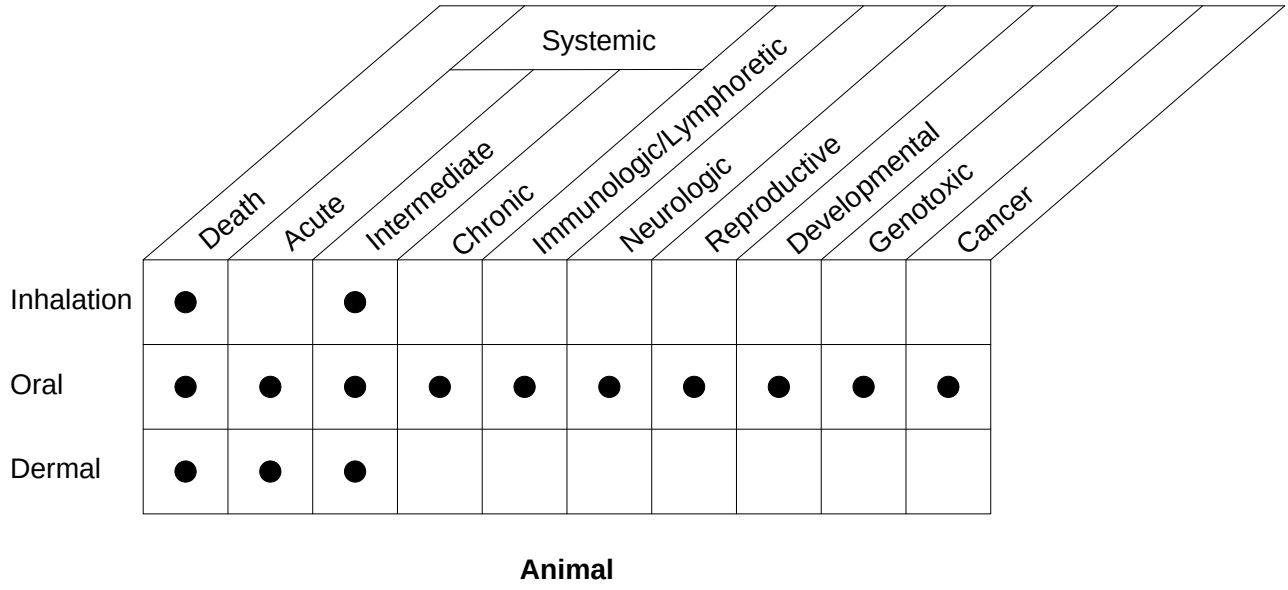
### 3.12.1 Existing Information on Health Effects of RDX

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to RDX are summarized in Figure 3-3. The purpose of this figure is to illustrate the existing information concerning the health effects of RDX. Each dot in the figure indicates that one or more studies provide

## Figure 3-3. Existing Information on Health Effects of RDX




**Human**




**Animal**

● Existing Studies

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 1989d), 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.

Case studies are available regarding systemic effects in humans following acute exposures to RDX via all three routes. One study in the workplace provides information on immunological and neurological effects following inhalation exposure for chronic periods (Hathaway and Buck 1977). Neurological effects have also been described following acute oral exposures to RDX (Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986).

Animal data on inhalation exposure is limited to one study. Oral animal data are available for all exposure durations and for all end points. Dermal data on death and systemic effects are available for animals exposed to RDX for acute and intermediate exposure periods.

### 3.12.2 Identification of Data Needs

**Acute-Duration Exposure.** The nervous system is one of the main targets for RDX toxicity in humans exposed by the inhalation (Hollander and Colbach 1969; Testud et al. 1996a) or oral (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986) routes, and animal studies involving oral exposure support this finding (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1985b, 2006). There is a small number of acute-duration animal studies and no studies that adequately examined potential systemic effects. Increases in occurrence of convulsions, tremors, and/or seizures were consistently observed in the available studies (Burdette et al. 1988; U.S. Army 1980b, 1985b, 1986d, 2006). In addition, decreases in growth were observed in the fetuses of rats exposed to lethal doses of RDX (U.S. Army 1986d). One animal study suggests that the skin is a target organ for RDX following dermal exposure (U.S. Army 1974). However, the use of solvents confounded the results. No acute inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. The available acute exposure data for

animals was adequate for the derivation of an MRL based on an increased incidence of convulsions/seizures/tremors (U.S. Army 2006). Further acute inhalation and oral studies on the developmental and neurological effects of RDX would be useful in determining levels that may cause harm to humans living near hazardous waste sites; these studies should also evaluate potential systemic effects.

**Intermediate-Duration Exposure.** No studies examining the toxicity in humans following intermediate-duration exposure to RDX were identified. No animal studies were identified examining RDX toxicity following inhalation exposure; thus, an intermediate-duration inhalation MRL could not be derived. Inhalation studies are needed to identify potential targets of toxicity and establish dose-response relationships; these studies would be useful in determining levels that may cause harm to humans who live near hazardous waste sites. The nervous system is the target organ for RDX toxicity in animals exposed by the oral route for intermediate periods (Levine et al. 1981, 1990; U.S. Army 1983a, 1985b, 2006; U.S. Navy 1974b; von Oettingen et al. 1949). The most consistently observed effect was convulsions, seizures, and tremors. Systemic effects (hematological and serum chemistry alterations), reproductive effects (testicular degeneration, possible decrease in male fertility), and developmental effects (decreases in growth and decreased viability) have also been observed. However, these effects have not been consistently observed across studies. Difference in the exposure route (dietary versus gavage) and RDX formulation (finely ground versus coarsely ground) may explain possible differences in the results; however, this has not been adequately assessed and additional oral exposure studies are needed to evaluate apparent study differences. An intermediate oral MRL based on an increased incidence of convulsions in rats was derived (U.S. Army 2006). Studies involving intermediate dermal exposure to RDX did not identify a target organ (U.S. Army 1974).

**Chronic-Duration Exposure and Cancer.** Only one human study was located for chronic-inhalation exposure. This study revealed no adverse health effects following chronic exposures to unknown levels of RDX in the air (Hathaway and Buck 1977). No animal studies concerning chronic inhalation exposure were located. No chronic inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. Therefore, further inhalation studies would be useful to identify target organs and define the potential for human health risks.

No human studies concerning chronic oral exposure were located. The most sensitive target organ for adverse effects in animals following chronic oral exposure is the nervous system; an increased occurrence of convulsions and seizures were observed in rats (U.S. Army 1983a). Mild adverse systemic effects

have also been observed in rats (U.S. Army 1983a) and mice (U.S. Army 1984c). A second chronic-duration study in rats (U.S. Navy 1976) did not find any adverse effects. An increased incidence of prostate gland inflammation was observed in rats exposed to RDX for 2 years (U.S. Army 1983a). The inflammation was observed at the lowest adverse effect level; it may have been secondary to a bacterial infection. Because the second rat study (U.S. Navy 1976) did not examine the prostate, the prostate effect could not be confirmed. Additional studies are needed to further evaluate the prostate as a potential target of RDX toxicity. These studies should include end points addressing immunotoxicity of chronic exposure to RDX. A chronic-duration oral MRL was derived for RDX based on the neurological effects observed in the U.S. Army (1983a) study. Only one human study was located for chronic dermal exposure (Sunderman 1944). This study reported dermatitis in workers exposed to RDX, but no dose levels were reported. No animal studies concerning chronic dermal exposure were located. Additional chronic oral and dermal studies would be useful to better define dose levels that may cause a risk to humans.

No studies are available regarding cancer in humans following any route of exposure. Increased incidences of combined hepatocellular adenomas and carcinomas were found in female mice orally exposed to RDX (U.S. Army 1984c). A re-evaluation of the histopathology slides from this study resulted in a re-classification of several of the tumors as nonneoplastic alterations (Parker et al. 2006). No increases in neoplastic lesions were observed in rat oral exposure studies (U.S. Army 1983a; U.S. Navy 1976). The risk of developing cancer by the inhalation or dermal routes has not been investigated. Further inhalation, oral, or dermal carcinogenicity studies would be useful to determine whether RDX poses a risk of cancer for humans.

**Genotoxicity.** Data from microbial mutagenicity studies using *S. typhimurium* and *S. cerevisiae* have consistently produced negative results (George et al. 2001; Lachance et al. 1999; Pan et al. 2007; U.S. Army 1977b, 1980b; Whong et al. 1980). Therefore, at this time, additional studies with RDX would probably not provide any new key information. Studies involving humans and mammalian species are few. The three mammalian studies available were negative for DNA damage in human fibroblasts (U.S. Army 1978b), dominant lethal mutations in rats (U.S. Army 1980b), and induction of micronuclei in bone marrow cells from mice (Reddy et al. 2005a). Additional studies of N-nitroso metabolites of RDX, such as MNX and TNX, would be valuable since N-nitroso compounds often yield genotoxicity. Research employing toxicogenomics or a combination of genetics, molecular biology, and bioinformatics may be able to uncover the molecular targets of RDX.

**Reproductive Toxicity.** No data are available on the reproductive toxicity of RDX in humans via inhalation, oral, or dermal routes of exposure. No inhalation or dermal studies are available for animals. An oral study in mice (U.S. Army 1984c) and one in rats (U.S. Navy 1976) revealed no histopathology in the ovaries, testes, or uterus. One oral study (U.S. Army 1983a) did reveal spermatic granulomas in the prostate of rats after 6 months of exposure and testicular degeneration in rats exposed for 1 year. This study also reported an increased incidence of suppurative inflammation of the prostate in rats exposed for 2 years; however, the inflammation was primarily observed in rats dying early and there is concern that the inflammation may be secondary to a bacterial infection rather than a primary effect of RDX. No pharmacokinetic data are available that can be used to determine whether the reproductive system is likely to be a target for RDX toxicity. Therefore, further studies to determine whether the prostate is indeed the most sensitive organ are important. A two-generation reproductive study in rats (U.S. Army 1980b) reported nonsignificant decreases in $F_0$ male fertility when the exposed males were mated with unexposed females or exposed females; additional studies are needed to confirm this effect.

**Developmental Toxicity.** No human studies on developmental effects are available for exposure to RDX via inhalation, oral, or dermal routes. No inhalation or dermal studies are available for animals. Two acute duration oral studies examined the potential developmental toxicity of RDX. Maternal deaths were observed in both studies at the highest dose tested (U.S. Army 1980b, 1986d). No increases in the occurrence of fetal malformations were observed (U.S. Army 1980b). One study reported a decrease in fetal weight and length at the dose level associated with maternal deaths and neurotoxicity. In a two-generation study, increases in the occurrence of stillbirths and decreases in pup survival were observed in the $F_1$ offspring of dams exposed to lethal doses; a decrease in pup body weights and increase in the incidence of renal cysts were observed in the $F_2$ pups (U.S. Army 1980b). The one available oral study in rabbits revealed no fetotoxicity (U.S. Army 1980b). No pharmacokinetic data are available that can be used to determine whether the developmental system is likely to be a target organ. Further developmental studies via the oral route are important to determine whether humans exposed to RDX at or near hazardous waste sites are at risk of experiencing adverse developmental effects.

**Immunotoxicity.** The only available immunological study in humans reveals no changes in the antinuclear antibodies of workers exposed to RDX in the air (Hathaway and Buck 1977). No other functional tests were performed. No histopathological alterations were found in the spleen, thymus, or lymph nodes of rats (Levine et al. 1990; U.S. Army 1980b, 2006) or mice (U.S. Army 1980b), or in the spleens of dogs (U.S. Navy 1974a; von Oettingen et al. 1949) or monkeys (U.S. Navy 1974b), after intermediate exposure via the oral route. In addition, no alterations in the proportion of cell surface

markers were observed in rats (U.S. Army 2006). A study by Levine et al. (1981) demonstrated mild leukocytosis. Further oral studies examining immune function would be useful to determine whether RDX adversely affects the immune system. In addition, inhalation and dermal studies would help determine whether exposure to RDX at or near hazardous waste sites would affect the human immune system.

**Neurotoxicity.** The nervous system is a major target organ for RDX toxicity. Seizures have been reported in humans exposed for acute periods by inhalation (Kaplan et al. 1965; Testud et al. 1996a), ingestion (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Kasuske et al. 2009; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986), or a combination of the inhalation and oral routes (Hollander and Colbach 1969; Ketel and Hughes 1972). Oral studies in animals have supported this finding for acute (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1980b, 1986d, 2006), intermediate (Sunderman 1944; U.S. Army 1983a, 2006; U.S. Navy 1974b; von Oettingen et al. 1949), and chronic (U.S. Army 1983a) exposure durations. Neurobehavioral alterations were observed in rats receiving a single gavage dose of RDX (U.S. Army 1985b), but not after repeated intermediate-duration exposure (U.S. Army 1985b, 2006). The conflicting results may be a reflection of when the behavioral tests were conducted, in relation to gavage dosing rather than a duration-related difference. Additional neurobehavioral function tests are needed to confirm the results observed in the acute study. More sensitive neurological tests in animals via inhalation, oral, or dermal routes would be helpful in establishing definite less serious LOAELs.

**Epidemiological and Human Dosimetry Studies.** There is one human study that tested blood chemistry and hematology in 70 workers exposed to an average of 0.28 mg/m$^3$ of RDX in the air (Hathaway and Buck 1977). All of the other human studies are case reports of individuals ingesting RDX (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986) or exposed to RDX dust (Kaplan et al. 1965; Testud et al. 1996a). No epidemiology studies are available for exposure in drinking water. If populations with appropriate exposures could be identified, it would be useful to conduct epidemiologic and human dosimetry studies to establish cause-and-effect relationships and to plan future monitoring of individuals living near hazardous waste sites.

## Biomarkers of Exposure and Effect.

***Exposure.*** Thus far, RDX in urine or blood is the only known biomarker of exposure to RDX. RDX has been measured in these media in cases of accidental ingestion of the chemical (Küçükardalĭ et al. 2003; Woody et al. 1986). There is no information regarding the metabolism of RDX in humans; therefore, monitoring of the blood and/or urine of RDX workers could help identify RDX-derived products that can be used as biomarkers in studies of populations living near sites where RDX has been found.

***Effect.*** There is no known sensitive biomarker for the effects of RDX. The most prominent effects are seizures in humans (Davies et al. 2007; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) or animals (Burdette et al. 1988; Schneider et al. 1977; U.S. Army 1983a; U.S. Navy 1974b; von Oettingen et al. 1949), but seizures can be evoked by a large number of substances and disease states. As mentioned previously, two studies of accidental poisoning with RDX measured levels of RDX in blood from the patients. However, additional studies are necessary to establish: (1) levels of RDX in blood that are associated with adverse neurological effects and (2) levels of exposure that are associated with specific levels of RDX in blood.

**Absorption, Distribution, Metabolism, and Excretion.** No studies are available regarding the toxicokinetics of RDX in humans. However, pulmonary and gastrointestinal absorption of RDX in humans can be inferred from reports of adverse health effects following exposure by these routes (Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) and from measurements of RDX in blood and urine after exposure in some studies (i.e., Küçükardalĭ et al. 2003; Woody et al. 1986). Relatively poor dermal absorption of RDX was reported in a study with excised human skin (Reddy et al. 2008); similar findings were reported in a study with excised pig skin (Reifenrath et al. 2002). No studies were located regarding the metabolism of RDX in humans. Analysis of blood and excreta from workers exposed to RDX could provide valuable information regarding the metabolism of RDX in humans. No inhalation toxicokinetic data were located in animals. Oral studies in rats indicate that mixing RDX with soil considerably reduces its bioavailability (Crouse et al. 2008). A recent study in miniature pigs showed that oral administration of RDX in carboxymethylcellulose and water results in almost complete absorption (Major and Reddy 2007). Earlier studies in rats provided information on some parameters of oral absorption, distribution, and elimination (Schneider et al. 1977, 1978). These studies did not show any preferential accumulation of RDX in tissues. RDX was found in

the brain of rat pups born to dams exposed to RDX during gestation (U.S. Army 2007b). Additional studies of the perinatal transfer of RDX in animals are needed, particularly to determine the relative contribution of gestational vs. lactational exposure. The metabolism of RDX has been studied in miniature pigs and the major metabolites have been characterized (Major and Reddy 2007). Since the main target for RDX appears to be the nervous system, additional studies of distribution of RDX and metabolites to different brain areas would be valuable. These studies should try to determine possible temporal correlations between the presence of RDX and/or metabolites in specific brain areas and the manifestation of clinical signs such as convulsive activity and seizures.

**Comparative Toxicokinetics.** The only comparative toxicokinetics data available are the results of dermal absorption studies in excised human and pig skin, which showed relatively poor absorption in both preparations (Reddy et al. 2008; Reifenrath et al. 2002). This suggests that pigs would probably be a good animal model for dermal absorption studies. As mentioned previously, analyses of blood and urine from subjects exposed to RDX during its manufacture or use or from individuals accidentally or intentionally exposed to high amounts of RDX could provide information on the metabolism of RDX in humans that can be compared with data collected from animals studies to establish which animal species serves as the best model for extrapolating results to humans. A PBPK model was developed that simulates disposition of RDX in the rat, swine, and humans (Krishnan et al. 2009; Sweeney et al. 2012; U.S. Army 2007a); this model was considered suitable for risk assessment.

**Methods for Reducing Toxic Effects.** There are no known mitigation measures specifically for RDX-induced toxicity, other than standard anticonvulsant therapy. Since gastrointestinal absorption of RDX seems to be quite slow (Küçükardalĭ et al. 2003), studies should focus on developing methods to accelerate its removal from the gastrointestinal tract to prevent RDX-induced adverse neurological effects. Washing the skin was reported to be effective in removing the chemical from the skin (Twibell et al. 1984). No data are available regarding adverse health effects of low-level, long-term exposure of humans to RDX; therefore, no specific mitigation studies can be proposed at this time for that exposure scenario.

**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 are limited data on potential age-related differences in the toxicity and toxicokinetics of RDX. Neurological effects, similar to those observed in adults, have been observed in a child accidentally ingesting RDX (Woody et al. 1986). However, the lack of dose information precludes determining whether children are more susceptible than adults. A study in deer mice found age-related differences in lethality (Smith et al. 2007). Additional animal studies are needed to evaluate whether there are potential differences in RDX toxicity between adults and children. These studies should include a wide-range of ages from birth through old age to assess whether there are differences in susceptibility as the nervous system matures.

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

The U.S. Army is concurrently developing a swine PBPK model, which can be extrapolated to humans.

No ongoing studies pertaining to RDX were identified in the Federal Research in Progress database (FEDRIP 2009).

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# 4. CHEMICAL AND PHYSICAL INFORMATION

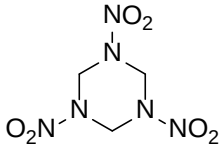
## 4.1 CHEMICAL IDENTITY

RDX is a nitramine produced mainly for use in explosives (HSDB 2009). Information regarding the chemical identity of RDX is located in Table 4-1.

## 4.2 PHYSICAL AND CHEMICAL PROPERTIES

RDX is a white crystalline solid. Information regarding the physical and chemical properties of RDX is located in Table 4-2. Pure RDX is a highly explosive compound that can be initiated by impact, temperature, and friction (Akhavan 2004; Boileau et al. 2009; HSDB 2009). RDX is toxic by inhalation and dermal routes (Lewis 2007). Acrid fumes of nitrogen oxides may be released when heated to decomposition (HSDB 2009; Lewis 2000).

## Table 4-1. Chemical Identity of RDX

| Characteristic | Information | Reference |
|---|---|---|
| Chemical name | RDX | HSDB 2009 |
| Synonym(s) | Cyclonite; hexogen; cyclotrimethylenetrinitramine; hexogen 5W;T4; hexahydro-1,3,5-trinitro-1,3,5-triazine; 1,3,5-triaza-1,3,5,-trinitrocyclohexane; 1,3,5-trinitrohexahydro-1,3,5-triazine; cyclotrimethylenenitramine; hexolite; S-triazine, hexahydro-1,3,5-trinitro-; 1,3,5-triazine, hexahydro-1,3,5-trinitro-; 1,3,5-triazine, perhydro, 1,3,5-trinitro-; trimethylenetrinitramine; sym-trimethylene trinitramine; 1,3,5-trinitrohexahydro-S-triazine; 1,3,5-trinitroperhydro-1,3,5-triazine; 1,3,5-trinitro-1,3,5-triazacyclohexane; trinitrotrimethylenetriamine | HSDB 2009 |
| Registered trade name(s) | No data | |
| Chemical formula | $C_3H_6N_6O_6$ | HSDB 2009 |
| Chemical structure |  | O'Neil et al. 2006 |
| Identification numbers: | | |
| CAS registry | 121-82-4 | HSDB 2009 |
| RTECS | XY9450000 | RTECS 2009 |
| EPA hazardous waste | No data | |
| OHM/TADS | No data | |
| DOT/UN/NA/IMDG shipping | UN0072; UN0391; UN0483; IMO1.1; DOT Explosive 1.1D | HSDB 2009; Lewis 2000 |
| HSDB | 2079 | HSDB 2009 |
| NCI | No data | |

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; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

## Table 4-2. Physical and Chemical Properties of RDX

| Property | Information | Reference |
|---|---|---|
| Molecular weight | 222.26 | Merck 1989 |
| Color | White | Akhavan 2004 |
| Physical state | Crystalline solid | Akhavan 2004 |
| Melting point | 204–206 °C | Boileau et al. 2009; Merck 1989 |
| Boiling point | Decomposes | U.S. Army 1991 |
| | Decomposition temperature: 213 °C | Akhavan 2004 |
| Density at 20 °C | 1.82 g/mL | Merck 1989 |
| Odor | No data | |
| Odor threshold: | | |
| Water | No data | |
| Air | No data | |
| Solubility: | | |
| Water at 20 °C | 38.4–38.9 mg/L; 60 mg/L | U.S. Army 1983b, 1991 |
| Organic solvents | Slightly soluble in methanol, ether, ethyl acetate, glacial acetic acid | Merck 1989 |
| Partition coefficients: | | |
| Log $K_{ow}$ | 0.87 | HSDB 2009; PHYSPROP 2009 |
| Log $K_{oc}$ | 1.80[a] | U.S. Army 1987a |
| Vapor pressure | | |
| At 20 °C | $1x10^{-9}$ mm Hg (Torr) | U.S. Army 1987a |
| At unidentified temperature | 0.05 Pa ($3.8x10^{-4}$ mm Hg) | Boileau et al. 2009 |
| Henry's law constant at 25 °C | $2.0x10^{-11}$ atm-$m^3$/mol[b] | PHYSPROP 2009 |
| Autoignition temperature | No data | |
| Flashpoint | No data | |
| Flammability limits | No data | |
| Explosive limits | Explosion may be prompted by sudden shock, high temperature, or combination of both | HSDB 2009 |

[a]Calculated value
[b]Estimated value

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

## 5.1 PRODUCTION

No information is available in the Toxic Release Inventory (TRI) database on facilities that manufacture or process RDX because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2010).

RDX is produced by nitrolysis of hexamine with nitric acid (HSDB 2009; Lewis 2007). In the Bachmann process, used in the United States, hexamine is reacted with nitric acid, ammonium nitrate, glacial acetic acid, and acetic anhydride (Boileau et al. 2009; Budavari and O'Neil 1989; HSDB 2009; U.S. Army 1978c, 1986e). The crude product is filtered and recrystallized to form RDX (U.S. Army 1986a). The Woolrich process, typically used in the United Kingdom and France, does not use acetic anhydride. The raw materials consist of hexamine and 98–99% nitric acid; however, this complex exothermic reaction is not completely understood (Boileau et al. 2009).

Another process that has been used to manufacture RDX by the direct nitration of HMX has not yielded a percentage of RDX as high as that produced in the Bachmann process (Budavari and O'Neil 1989; U.S. Army 1978c).

Production of RDX peaked in the 1960s when it was ranked third in explosive production by volume in the United States (U.S. Army 1986e). The average volume of RDX produced from 1969 to 1971 was 15 million pounds per month. However, production of RDX decreased to a yearly total of 16 million pounds for 1984.

RDX is not produced commercially in the United States (HSDB 2009). Current production in the United States is limited to military use at the Holston Army Ammunition Plant in Kingsport, Tennessee (SRI 2009). In the past, several Army ammunition plants, such as Louisiana (Shreveport, Louisiana), Lone Star (Texarkana, Texas), Iowa (Middletown, Iowa), and Milan (Milan, Tennessee), may have also handled and packaged RDX (U.S. Army 1986e). In 1980, RDX was produced at five facilities in the United States, including Borden (Fayetteville, North Carolina), Hooker (North Tonawanda, New York), Plastics Engineering (Sheboygan, Wisconsin), Tenneco (Fords, New Jersey), and Wright Chemical

(Riegelwood, North Carolina). U.S. capacity in 1980 was 119 million pounds per year (CMR 1980). In 2006, 6.9 million pounds were produced at Holston Army Ammunition Plant (EPA 2006c).

## 5.2 IMPORT/EXPORT

No information is available regarding the import or export of RDX.

## 5.3 USE

RDX is a nitramine explosive compound (Turley and Brewster 1987) that can be utilized as a propellant, gunpowder, or high explosive depending on the initiation type (Boileau et al. 2009). RDX has both military and civilian applications. As a military explosive, RDX can be used alone as a base charge for detonators or mixed with another explosive such as TNT to form cyclotols, which produce a bursting charge for aerial bombs, mines, and torpedoes (HSDB 2009; Lewis 2000; Sax and Lewis 1989; Stokinger 1982). As an explosive, RDX is one and a half times more powerful than TNT and is easily initiated with mercury fulminate (Lewis 2007). Common military uses of RDX have been as an ingredient in plastic bonded explosives or plastic explosives, which have been used as explosive fill in almost all types of munition compounds (Gibbs and Popolato 1980; HSDB 2009). The plasticized form of RDX, composition C-4, contains 91% RDX, 2.1% polyisobutylene, 1.6% motor oil, and 5.3% 2-ethylhexyl sebacate (Turley and Brewster 1987). Combinations of RDX and HMX, another explosive, have been the chief ingredients in approximately 75 products (U.S. Army 1978c).

## 5.4 DISPOSAL

Waste water treatment sludges resulting from the manufacture of RDX are classified as hazardous wastes and are subject to EPA regulations (EPA 1990a). For more information on regulations that apply to RDX, see Chapter 8.

Propellants and explosives have been disposed of through burning, decomposition, re-use, and recovery (Bohn et al. 1997). Byproducts of military explosives such as RDX have also been openly burned in many Army ammunition plants in the past. There are indications that, in recent years, as much as 80% of waste munitions and propellants have been disposed of by incineration (U.S. Army 1986a). Wastes containing RDX have been incinerated by grinding the explosive wastes with a flying knife cutter and spraying the ground material with water to form a slurry. The types of incineration used to dispose of waste munitions containing RDX include rotary kiln incineration, fluidized bed incineration, and

pyrolytic incineration (U.S. Army 1986a). The primary disadvantage of open burning or incineration is that explosive contaminants are often released into the air, water, and soils (U.S. Army 1986c). Munitions such as RDX have also been disposed of in the past by dumping into deep seawater (Hoffsommer and Rosen 1972).

RDX wastes found in soils and sediments have been degraded in composts using substances such as hay, horse feed, sewage sludge, wood shavings or sawdust, animal manure, and fruit and vegetable wastes (Greist et al. 1993; Gunderson et al. 1997; U.S. Army 1986b; Williams et al. 1992). In a mechanically stirred amended compost, the concentration of RDX in soil was reduced from <800 to 39 mg/kg after 44 days (Griest et al. 1993). RDX in contaminated soil from a dry explosives washout lagoon decreased from 884 to <2.9 mg/kg after 6 months using a 70% organic compost (Gunderson et al. 1997). RDX has been removed from munitions waste waters and contaminated groundwater by activated carbon columns (Bricka and Sharp 1992; U.S. Army 1987c; Wujcik et al. 1992). No RDX was detected when contaminated groundwater containing 487 µg/L of RDX was passed through granular activated carbon (GAC) columns at a loading rate of 7.11 gpm/ft, a flow rate of 0.7 gpm, and an empty-bed contact time of 4.2 minutes (Wujcik et al. 1992). Once carbon columns were saturated with explosive, they were traditionally destroyed by open burning. Since this practice is no longer allowed in many areas, other disposal alternatives for spent carbons, such as thermal reactivation for reuse, oxidative incineration with ash burial, and thermal deactivation with carbon burial, have been investigated (U.S. Army 1987c). In a feasibility study, ultraviolet irradiation was found to provide effective treatment of RDX-contaminated groundwater (Bricka and Sharp 1992).

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# 6. POTENTIAL FOR HUMAN EXPOSURE

## 6.1 OVERVIEW

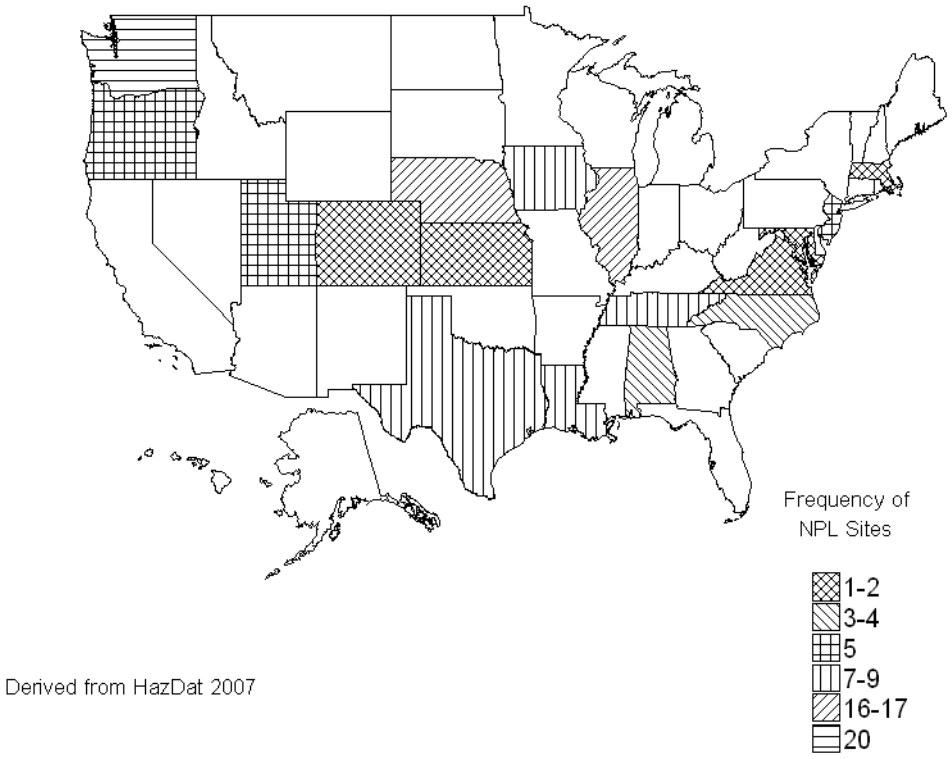
RDX has been identified at 31 out of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for RDX is not known. The frequency of these sites can be seen in Figure 6-1.

RDX is a military explosive produced by the nitrolysis of hexamine with nitric acid (Boileau et al. 2009). It is a synthetic compound and is not known to exist in nature. Effluents and emissions from ammunition plants are responsible for the release of RDX into the environment (Pennington and Brannon 2002; U.S. Army 1984a). RDX is expected to exist as a particulate in the atmosphere. When released to water, RDX is subject to photolysis (half-life of 9–13 hours). Photoproducts include formaldehyde and nitrosamines (U.S. Army 1980a). Alkaline hydrolysis can also occur (Balakrishnan et al. 2003; Heilmann et al. 1996). RDX undergoes biodegradation in water and soil under anaerobic conditions (Funk et al. 1993; Pennington and Brannon 2002; U.S. Army 1984f). Its biodegradation products include MNX; DNX; TNX; hydrazine; 1,1-dimethyl-hydrazine, 1,2-dimethyl-hydrazine; formaldehyde; and methanol (McCormick et al. 1981). RDX is mobile in soil, and can leach into groundwater (U.S. Army 1980c), and can be transported from soils or water to terrestrial and aquatic plants (Best et al. 1999; Harvey et al. 1991, 1997; Pennington and Brannon 2002; Simini and Checkai 1996).

RDX has been identified in environmental samples, primarily near army munition depots (Bishop et al. 1988; Dacre 1994). Indoor air samples collected at ammunition plants were found to contain RDX in concentrations ranging from 0.032 to 60 mg/m$^3$ (Bishop et al. 1988; U.S. Army 1975). In water, RDX has been identified in a variety of groundwater samples from ammunition plants in the United States (<1–14,100 μg/L) and Germany (21–3,800 μg/L) (Bart et al. 1997; Best et al. 1999; Godejohann et al. 1998; Steuckart et al. 1994; U.S. Army 1988). Sediment samples from Army depots have been found to contain RDX in concentrations ranging from <0.1 to 3,574 mg/kg (Simini et al. 1995; Sunahara et al. 1999; U.S. Army 1988) and in composts prepared from contaminated sediments (>2.9–896 mg/kg) (Griest et al. 1995; Gunderson et al. 1997). Additionally, RDX was indentified in plant species irrigated with or grown in contaminated water (<20–3,196 μg/L) (Best et al. 1999; Pennington and Brannon 2002).

For the general population, exposure to RDX is primarily limited to areas around ammunition plants and military installations where it is manufactured, converted to munitions, packed, loaded, or released

## Figure 6-1. Frequency of NPL Sites with RDX Contamination



through the demilitarization of antiquated munitions (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1984a, 1984f). The most likely route of exposure is ingestion of contaminated drinking water or agricultural crops irrigated with contaminated water (Harvey et al. 1991, 1997; Simini and Checkai 1996). Dermal contact with soil containing RDX or inhalation exposure of contaminated particulate matter produced during incineration of RDX-containing waste material are also possible routes of exposure. Occupational exposure to RDX can occur when workers handle RDX at Army ammunition plants (Hathaway and Buck 1977; Kaplan et al. 1965). According to the National Occupational Exposure Survey (NOES) of 1981–1983 conducted by NIOSH, the estimated number of workers potentially exposed to RDX in the United States was 488 (NIOSH 1990).

Since RDX releases are not required to be reported under SARA Section 313, there are no data on RDX in the Toxics Release Inventory (TRI 1993).

## 6.2 RELEASES TO THE ENVIRONMENT

### 6.2.1 Air

There is no information on releases of RDX to the atmosphere from manufacturing and processing facilities because these releases are not required to be reported (EPA 1997). However, all emissions are evaluated under Title V of the 1990 federal Clean Air Act Amendments within each state's Title V programs. RDX emissions from the manufacturing process are considered insignificant under the Title V air pollution control permits for facilities because they are contained systems. Thus, emission quantities are such that dispersion from the facilities is unlikely to be detectable by ambient monitoring.

RDX can enter the air through the release of contaminated particulate matter formed during the incineration of RDX-containing mixtures (U.S. Army 1984a). RDX can also enter the air through evaporation from aquatic effluent streams or waste storage lagoons (U.S. Army 1984a).

### 6.2.2 Water

There is no information on releases of RDX to the water from manufacturing and processing facilities because these releases are not required to be reported (EPA 1997). Water discharges from RDX manufacturing and processing facilities are regulated by the National Pollutant Discharge Elimination System (NPDES) permit managed by each state's NPDES program. The monitoring methodology may vary from state to state.

RDX can be released to water in waste discharge effluents from ammunition production, formulation, manufacturing, loading, assembly, and packing, and through the demilitarization and disposal of antiquated munitions (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1984a, 1984f).

### 6.2.3 Soil

There is no information on releases of RDX to the soil from manufacturing and processing facilities because these releases are not required to be reported (EPA 1997). Releases to soil are generally confined to manufacturing facilities and points of use such as firing ranges. These sites are monitored by the Department of the Army as well as state and federal environmental regulatory authorities under several environmental programs such as CERCLA, RCRA, Emergency Planning and Community Right-to-Know Act (EPCRA). Response activities include monitoring, cleanup, and land use controls as determined approporiate.

Manufacturing, packing, and use of RDX have often resulted in contamination of soil. RDX can enter soil by leaching from waste lagoons and from improper disposal of contaminated sludge (U.S. Army 1984a). RDX can also enter the soil from spills during manufacture, transportation, and storage. Releases can also occur from the settling of airborne particulates from manufacturing and demilitarization practices such as incineration onto soil surfaces (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1984a).

## 6.3 ENVIRONMENTAL FATE

### 6.3.1 Transport and Partitioning

RDX is expected to exist in the particulate phase in the atmosphere. The solubility of RDX in water is low to negligible (Budavari and O'Neil 1989). The following water solubility values have been reported: 21.8–21.9 mg/L at 10 °C, 38.4–38.9 mg/L at 20 °C, 59.7 mg/L at 25 °C, and 66.7–67 mg/L at 30 °C (U.S. Army 1983b; Yalkowsky and He 2003). RDX is slightly soluble in methanol, ether, ethyl acetate, and glacial acetic acid (Budavari and O'Neil 1989). The Henry's law constant for RDX is approximately $2x10^{-11}$ atm-$m^3$/mol (PHYSPROP 2009), indicating that volatilization from water or moist soil surfaces is expected to be a slow process.

The soil adsorption coefficients normalized to organic carbon content ($K_{oc}$) for RDX range from 42 to 167 (U.S. Army 1980c). These $K_{oc}$ values are indicative of moderate-to-high mobility in soil (Swann et al. 1983); therefore, RDX can be expected to leach into groundwater. Experimental data have shown that RDX is not readily bound or retained in soil as evidenced by its early breakthrough in column leachates (U.S. Army 1985a). A lysimeter study of the migration of RDX in soil showed that RDX was found in leachate from the soil columns (U.S. Navy 1982). Based on these $K_{oc}$ values and the experimental data, adsorption to sediment and particulate matter in the aquatic environment should not be significant (U.S. Army 1980a). Although RDX does not significantly adsorb to sediment, greater adsorption occurs with an increase in organic matter or clay content (U.S. Army 1980a). However, the clay content seems to be more important than organic matter content in influencing the amount of RDX adsorbed (U.S. Army 1980a). In a study sponsored by the U.S. Army Medical Research and Development Command (USAMRDC), the adsorption rate constant of RDX in soil was found to be low ($K_d$ of <1 mg/g). The adsorption constant was linearly correlated with a combination of soil properties, such organic carbon and clay content, pH, and cation exchange capacity (U.S. Army 1993a). Adsorption to soil was measured using samples from the Louisiana Army Ammunition Plant. RDX was retained on a bentonite/sand column with a 90% recovery after 11 pore volumes. Retardation of RDX by fine-silty soils was limited (Selim et al. 1995). It appears that sorption of RDX in soils is not solely the result of hydrophobic partitioning of RDX to the organic carbon phase of the soils.

The logarithm of the octanol/water partition coefficient (log $K_{ow}$) is a useful preliminary indicator of potential bioaccumulation of a compound. The log $K_{ow}$ for RDX was estimated to be 0.87 (PHYSPROP 2009), indicating that RDX is not very lipid soluble and therefore has a low potential for bioaccumulation in aquatic species. Experimental bioconcentration factors in edible tissue for bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus punctatus*), and fathead minnow (*Pimephales promelas*) were 1.9–6.4, 1.2–5.5, and 1.4–5.9, respectively (U.S. Army 1984a). These factors indicate that bioaccumulation in aquatic organisms is not an important fate process.

Data indicate that RDX can be taken up by both terrestrial and aquatic plants (Best et al. 1999; Harvey et al. 1991, 1997; Pennington and Brannon 2002; Simini and Checkai 1996; U.S. Army 1990a). Studies of bean plants grown in 10 ppm RDX hydroponic solutions and exposed for 1 or 7 days indicated that uptake of RDX readily occurred. Following uptake, translocation of the compounds to the aerial tissue occurred, resulting in foliar concentrations of 20 and 97 ppm for the 1- and 7-day exposures, respectively. Metabolism of RDX to polar metabolites was observed in plants exposed for 7 days (Harvey et al. 1991). Additional studies of hydroponic plant-culture systems indicated that RDX (1–10 ppm) was also absorbed

by the roots of blando brome and wheat and that plant absorption was concentration-dependent (U.S. Army 1990a). In a simulation of field conditions, uptake of RDX to lettuce leaves, corn stover, and alfalfa shoots correlated to levels of RDX (2, 18, and 90 ppb) in the irrigation water (Simini and Checkai 1996). Submerged aquatic plants, including Elodea, pondweed, and water star-grass, grown using sediment and contaminated groundwater containing 1,529 µg/L from the Milan Ammunition Plant in Milan, Tennessee had RDX concentrations of 976, 42, and 1,496 µg/L, respectively, after 13 days. The emergent plant species, parrot-feather, sweet-flag, reed canary grass, and wool-grass contained RDX at 3,196, 1,156, 704, and <20 µg/L, respectively (Best et al. 1999). When grown in soil contaminated with 58 mg/kg RDX, lettuce was found to contain 1,200 mg/kg of RDX, while nutsedge, tomato fruit, corn kernels, and corn stover contained RDX at concentrations of 62, 7, 6, and 56 mg/kg, respectively (Pennington and Brannon 2002). For plants grown in soils containing 10 ppm RDX over a period of 60 days, the extent of plant uptake was found to be dependent both on soil type and plant species (Cataldo et al. 1993). RDX was transported unchanged from soils to plants and the plant uptake increased as the organic matter content of soil decreased. In bush bean plants, RDX was mostly concentrated in leaves and seed, with less found in roots, stems, and pods. In the case of wheat and blando brome, RDX mostly concentrated in leaves and roots, with very little or none in seeds (Cataldo et al. 1993). After plant uptake, RDX in storage tissues of plants (i.e., roots and stems) mostly metabolized to unidentified polar metabolites or nonextractable products, while RDX remained mostly unchanged (>50%) in leaves and seed tissues (Cataldo et al. 1993).

### 6.3.2 Transformation and Degradation

#### 6.3.2.1 Air

RDX is expected to exist in the particulate form in the atmosphere, and may be subject to removal from air by dry deposition. No data were located on photolysis of RDX in the atmosphere. However, it is expected that photolysis of RDX is an important fate process in the atmosphere since RDX absorbs ultraviolet wavelengths between 240 and 350 nm (U.S. Army 1986e) and it undergoes rapid photolysis in water (U.S. Army 1980a).

#### 6.3.2.2 Water

In a hydrolysis study of RDX in seawater (pH 8.1) at 25 °C, 11.6% of initial RDX hydrolyzed in 112 days (Hoffsommer and Rosen 1973). Other data found that RDX was stable to hydrolysis in an aqueous solution at a pH range normally found in natural waters (U.S. Army 1980a). Therefore, hydrolysis is not

expected to significantly influence the environmental fate of RDX. Hydrolysis can occur, however, under alkaline conditions. RDX underwent alkaline hydrolysis (pH 10) in the presence of water over 17 days. The approximate half-life for this reaction was about 7 days and was accompanied by the formation of the ring cleavage product 4-nitro-2,4-diazabutanal, as well as $NO_2^-$, $N_2O$, formaldehyde, and formic acid (Balakrishnan et al. 2003). Aqueous alkaline hydrolysis is thought to be a possible method of remediating RDX contaminated waste water (Heilmann et al. 1996).

The primary physical mechanism that degrades RDX in aqueous solutions is photolysis (U.S. Army 1986e). The range of ultraviolet wavelengths that produce photolytic reactions with RDX is generally between 240 and 350 nm (U.S. Army 1986e). RDX in waste water (23.9 mg/L) exposed to ultraviolet radiation decomposed with a half-life of 3.7 minutes (Burrows et al. 1984). Photolysis of an aqueous solution of RDX in natural sunlight is fairly rapid with an experimental half-life of 9–13 hours. Consequently, RDX is not expected to persist for a long period of time in clear, sunlit surface waters (U.S. Army 1980a). Formaldehyde and nitrosamines were identified as photoproducts. Nitrosamines may be of environmental importance because of their potential mutagenicity/carcinogenicity. Conversion to this product, however, occurs only to a limited extent since the product itself is photoreactive (U.S. Army 1980a). The rate of photodegradation under different environmental conditions is also dependent upon the nature of the water body itself. RDX was shown to degrade very slowly in dark, tea-colored lagoon waters at a Louisiana Army Ammunition Plant during a field study at this site (U.S. Army 1983b). The half-life for RDX was approximately 2,100 days in winter and 456 days in summer for a lagoon 50 cm deep (U.S. Army 1983b). The slow rate of degradation was attributed to the rapid attenuation of sunlight in the top layers of the water column, thereby preventing photons of radiation from reaching RDX, which was reported to be well mixed throughout the water column (U.S. Army 1983b).

The biodegradation of RDX has been studied under aerobic and anaerobic conditions. RDX did not undergo aerobic biodegradation using a variety of inocula and nutrients (Osmon and Klausmeier 1973). However, microbial degradation studies were carried out using water and sediment samples collected from the Holston River and the waste-water effluents from the Holston Army Ammunition Plant showed some degradation (U.S. Army 1980a). Only the addition of river sediments appeared to stimulate the aerobic biodegradation of RDX in samples of river water containing either 5.5 or 11.5 ppm of RDX. The half-life for the disappearance of RDX in water samples supplemented with sediment was approximately 7 days. A lag period of 2–3 weeks was observed before a noticeable degradation of RDX occurred. The results showed that biodegradation of RDX leads to mineralization of the molecule (U.S. Army 1980a). No degradation of RDX was observed during a 90-day aerobic experiment with RDX in the lagoon water

alone, with added yeast extract, or with 1% of bottom sediment (U.S. Army 1983b). Concentrations of RDX remained unchanged when cultures were inoculated with aerobic activated sludge and incubated aerobically. No RDX disappeared in uninoculated controls (McCormick et al. 1981).

Data are available indicating that biodegradation of RDX occurs under anaerobic conditions (U.S. Army 1984f; Crocker et al. 2006; Funk et al. 1993; Hawari et al. 2000; McCormick et al. 1981; Pennington and Brannon 2002; Walker and Kaplan 1992). RDX (50 or 100 μg/mL) disappeared rapidly from nutrient broth cultures inoculated with anaerobic sewage sludge and incubated anaerobically. Biodegradation of RDX was complete after 4 days (McCormick et al. 1981). The disappearance of RDX was accompanied by the appearance of several products identified as the mono-, di-, and trinitroso derivatives of RDX formed by sequential reductions of the nitro groups to nitroso groups (Crocker et al. 2006; Hawari et al. 2000; McCormick et al. 1981; Walker and Kaplan 1992). Anaerobic biodegradation products included MNX; DNX; TNX; hydrazine; 1,1-dimethyl-hydrazine; 1,2-dimethyl-hydrazine; formaldehyde; and methanol. The nitroso intermediates are known to be hazardous. Both 1,1- and 1,2-dimethylhydrazine, as well as hydrazine, are known mutagens and/or carcinogens (McCormick et al. 1981), but may be found naturally in the environment (e.g., certain mushrooms).

After an incubation period of 5 days, 97% of RDX was anaerobically degraded by a mixed population of purple photosynthetic bacteria of the genera Chromatium, Rhodospirillum, and Rhodopseudomonas, and possibly others (U.S. Navy 1973). Sixty percent of RDX was anaerobically degraded by Chromatium alone (U.S. Navy 1973). These photosynthetically active cultures, which do not release oxygen, were supplemented with sodium acetate and ammonium chloride. It was hypothesized that RDX was not actually metabolized, but rather was being reduced and modified as a result of the active electron transfer brought about by the anaerobic photosynthetic activity of the organisms. Data indicate that hydrogen can be the sole electron donor in the anaerobic degradation of RDX (Beller 2002). A proposed pathway for the degradation of RDX involves reductions leading to destabilization, ring cleavage, and mineralization. Degradation intermediates are much more susceptible to degradation under anaerobic conditions than under aerobic conditions (Pennington and Brannon 2002).

RDX (13 ppm) in lagoon waste water at the Louisiana Army Ammunition Plant did not undergo anaerobic degradation for approximately 90 days with yeast extract repeatedly added as a nutrient (U.S. Army 1983b). The RDX concentration fell to 2.9 ppm at day 90 and to 1.4 ppm at day 92. The authors reported that the repeated addition of yeast extract acclimated RDX-utilizing organisms. The RDX-

acclimated organisms then degraded 9.1 ppm of RDX 93% after 5 days of anaerobic incubation (U.S. Army 1983b).

#### 6.3.2.3 Sediment and Soil

Three soils containing 0.5–7.2% organic matter were amended with 60 ppm (mg/kg) RDX and incubated for 60 days under aerobic conditions (Cataldo et al. 1993). After 60 days, >95% were extractable and remained unchanged as parent RDX; only <2% remained nonextractable in the soils. No significant transformation products of RDX were observed in the soils. RDX was not biodegraded after 56 days following addition to three soil samples (Grant et al. 1995). RDX, present at 30 ppm in soil cultures containing added potato starch as an additional carbon source, was not degraded after 24 days (Funk et al. 1993). These results indicate that RDX may not be easily amenable to aerobic biodegradation in soils.

Significant biotransformation, however, may occur under certain conditions. The degradation of pink water compounds in soil was studied (U.S. Army 1985a). Pink water is a generic term used for colored waters that may contain some explosive compounds, including RDX. A simulated pink water containing RDX (30 mg/L) was continuously applied to a series of soil columns at different flow rates, with and without carbon supplementation. The columns were inoculated with combined samples of micro-organisms from activated sludge, anaerobic sludge digest, and garden soil. Concentrations of RDX and biotransformation products were monitored on a weekly basis. There appeared to be a significant decrease in RDX recovery in the leachate of the column with slow and fast flow with carbon supplement, indicating microbial activity. The mononitroso derivative, MNX, and the dinitroso derivatives of RDX were identified in the leachate of the column with fast flow (100 mL/day) and carbon supplement (2.0 g/L glucose). MNX was also identified in the leachates from the columns with slow flow (40 mL/day) with and without carbon supplement (U.S. Army 1985a). Since the nitroso derivatives are intermediates in the anaerobic biodegradation of RDX in aqueous systems (Walker and Kaplan 1992), it is likely that the observed products resulted from anaerobic biodegradation of RDX. The authors reported that land treatment or land farming of pink water should not be considered as a treatment option for pink water. Hazardous biotransformation intermediates and unchanged concentrations of some of the pink water compounds would contaminate groundwater and soil. RDX, present at 30 ppm in anaerobic soil cultures containing added potato starch as an additional carbon source, were totally degraded after 24 days (Funk et al. 1993).

Douglas et al. (2009) added 20 mL of a solution containing 2.3 mg/L RDX to aqueous slurries containing pristine soils and soils fractured to simulate the effects of detonation. After 92 days, the measured concentration was approximately 1.3–2.2 mg/L in the slurries containing the fractured soils and virtually unchanged in the slurries containing the pristine soil. The authors suggested that the observed decrease in aqueous concentration in the fractured soil could be caused by enhanced adsorption to fractured soil particle surfaces or enhanced transformation in the presence of the fractured soil particles or a combination of both.

## 6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to RDX depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of RDX 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 RDX 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 RDX in a variety of environmental media are detailed in Chapter 7.

### 6.4.1 Air

No data are available regarding levels of RDX in outdoor air. However, indoor air samples collected at Holston Army Ammunition Plant in Kingsport, Tennessee in 1974 contained RDX levels ranging from not detected (<0.5 mg/$m^3$ [4.5 ppm]) to 60 mg/$m^3$ (546 ppm) (U.S. Army 1975). A more recent study found that RDX was detected in only one of eight indoor air samples taken from the incorporation area of Holston Army Ammunition Plant in 1986; the concentration in this sample was 0.032 mg/$m^3$ (0.29 ppm) in the particulate fraction (Bishop et al. 1988).

### 6.4.2 Water

Seawater samples taken in 1971 from a munitions dumping area 85 miles west of Cape Flattery, Washington, and similar samples taken 172 miles south-southeast of Charleston, South Carolina, were analyzed for RDX (U.S. Navy 1972). No RDX was found in any of the samples examined (detection limit of 5 ppt). RDX was found on-site at the Savanna Army Depot in Illinois in surface water samples at a maximum reported concentration of 36.9 ppm (Agency for Toxic Substances and Disease Registry

1989c). The Savanna Army Depot is on the NPL. It was an Army munitions plant engaged in munitions renovation, loading, demolition, and burning, which was closed in 2000.

Onsite groundwater sampling at the Milan Army Ammunition Plant near Milan, Tennessee identified RDX at concentrations ranging from not detected to 11.24 ppm (detection limit not reported) (Agency for Toxic Substances and Disease Registry 1989b). Filtered groundwater samples from the Milan Army Ammunition Plant contained RDX at a concentration of 1,443 ppb. Filtration reduced RDX concentration in the water samples by 27% (Best et al. 1999). U.S. Army (2011) listed a range of detectable concentrations of 50–18,000 ppb in groundwater samples and 80–120 ppb in surface water samples taken from Milan Army Ammunition Plant. Groundwater samples from the Umatilla Army Depot Activity, a munitions storage and handling depot in Hermiston, Oregon and the Naval Submarine Base Bangor in Bangor, Washington contained RDX in concentrations ranging from <20 to 8,160 ppb (Bart et al. 1997).

Groundwater samples from monitoring and extraction wells at the Naval Base Kitsap at Bangor NPL site in Kitsap County, Washington, were collected from May 1994 to August 2004. Concentrations of RDX in the samples from a 12-acre Bangor Ordnance Disposal site (Site A) ranged from 0.19 to 1,000 ppb in perched zone monitoring wells, from 0.19 to 550 ppb in shallow aquifer monitoring wells, and from 0.4 to 660 µg/L in extraction wells (shallow aquifer). RDX concentrations at the site of a former waste water lagoon and overflow ditch (Site F) in groundwater from a shallow aquifer ranged from 0.95 to 3,800 ppb (U.S. Navy 2005).

RDX was identified in environmental samples at Cornhusker Army Ammunition Plant and Louisiana Army Ammunition Plant army bases (Dacre 1994). Maximum concentrations of RDX detected in water at the Cornhusker Army Ammunition Plant (Nebraska) were 307 and 371 ppb from on- and off-site wells, respectively (Agency for Toxic Substances and Disease Registry 1989a). A plume of RDX-contaminated groundwater, which stretched 6.5 km, was found near the Cornhusker Army Ammunition Plant. The concentrations ranged from 9 to >100 ppb (Spalding and Fulton 1988). A maximum concentration of 95 ppb in groundwater was reported by U.S. Army (2011) for the Cornhusker Army Ammunition Plant The Louisiana Army Ammunition Plant is a shell manufacturing and explosives load, assembly, and pack facility (U.S. Army 1988). From 1951 to 1980, waste waters were trucked to and discharged into a series of artificial leaching pits, which resulted in contamination of soil, sediments, and groundwater. Levels of RDX measured in groundwater at the Louisiana Army Ammunition Plant ranged from 1.3 to 14,100 ppb (U.S. Army 1988). U.S. Army (2011) reported a maximum groundwater concentration of 13,200 ppb at

this facility. U.S. Army (2011) also reported ranges of groundwater concentrations of 0.0087–86.4 ppb at Aberdeen Proving Ground and 3.3–13,000 ppb at Iowa Army Ammunition Plant. The surface water concentrations ranged from 0.73 to 7.6 ppb at Aberdeen Proving Ground and 4.4 to 249 ppb at Iowa Army Ammunition Plant.

RDX was identified in a water sample obtained from a military training site in Germany at 21 ppb (Godejohann et al. 1998). Two contaminated water samples from the area of a former explosive production plant at Elsnig in Saxony, Germany contained RDX at concentrations of 2,380–3,800 and 310–400 ppb, with the exact concentrations dependent upon the method of detection (Steuckart et al. 1994).

### 6.4.3 Sediment and Soil

Ocean floor sediment samples taken in 1971 from a munitions dumping area 85 miles west of Cape Flattery, Washington, and similar samples taken 172 miles south-southeast of Charleston, South Carolina, were analyzed for RDX (U.S. Navy 1972). No RDX was found in any of the sediment samples analyzed. RDX was found onsite at the Savanna Army Depot in Illinois in soil samples at a maximum concentration of 12.3 ppm (Agency for Toxic Substances and Disease Registry 1989c). RDX was found at the Louisiana Army Ammunition Plant in soil and drainage sediments at concentrations ranging from <5 to 602 mg/kg (U.S. Army 1988). RDX was identified in a composite soil sample at a concentration of 130.5 mg/kg. The sample was composed of topsoil samples from the site of an explosives factory (Sunahara et al. 1999). Soils collected from the Joliet Army Ammunition Plant in Joliet, Illinois contained RDX in concentrations ranging from <0.1 to 3,574 mg/kg (Simini et al. 1995). RDX was identified in environmental samples at Cornhusker Army Ammunition Plant and Louisiana Army Ammunition Plant (Dacre 1994).

RDX was identified in compost at 884 mg/kg. The compost was prepared using contaminated sediments from the Umatilla Army Depot Activity in Hermiston, Oregon (Gunderson et al. 1997). Griest et al. (1995) identified RDX in dry compost prepared using soils from Umatilla in concentrations ranging from >2.9 to 896 mg/kg.

U.S. Army (2011) reported range of detectable soil concentrations of 5.45–890 mg/kg at Cornhusker Army Ammunition Plant, 0.587–3,300 mg/kg at Milan Army Ammunition Plant, 980 mg/kg at Aberdeen

Proving Ground, and 2.5–75,000 mg/kg at Iowa Army Ammunition Plant; sediment samples from Iowa Army Ammunition Plant contained 0.363–14,100 mg/kg RDX.

### 6.4.4 Other Environmental Media

Ocean floor fauna samples (rat tail fish and sea cucumbers) taken in 1971 from munitions dumping areas in the Atlantic and Pacific Oceans contained no apparent RDX residues (detection limit of 0.123 µg/kg) (U.S. Navy 1972).

Agricultural crops irrigated with contaminated water have been found to contain RDX. In a laboratory study simulating field conditions, uptake of RDX to lettuce leaves, corn stover, and alfalfa shoots correlated to levels of RDX in the irrigation water (2, 18, and 90 ppb). RDX did not significantly concentrate in tomatoes, bush bean seeds and pods, radish roots, and soybean seeds (Simini and Checkai 1996). Submerged aquatic plants, including Elodea, pondweed, and water star-grass, grown using sediment and contaminated groundwater containing 1,529 µg/L from the Milan Ammunition Plant in Milan, Tennessee had RDX concentrations of 976, 42, and 1,496 µg/L, respectively, after 13 days. The emergent plant species, parrot-feather, sweet-flag, reed canary grass, and wool-grass contained RDX at 3,196, 1,156, 704, and <20 µg/L, respectively (Best et al. 1999). When grown in soil contaminated with 58 mg/kg RDX, lettuce was found to contain 1,200 mg/kg of RDX, while nutsedge, tomato fruit, corn kernels, and corn stover contained RDX at concentrations of 62, 7, 6, and 56 mg/kg, respectively (Pennington and Brannon 2002).

## 6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

For the general population, exposure to RDX is most likely limited to areas around Army ammunition plants where RDX is manufactured, converted to munitions, or released through the demilitarization of antiquated munitions (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1984a, 1984f). Two surveys of public places, including taxis, trains, and airplanes, hotels, and private homes, rarely detected RDX (Crowson et al. 1996; Cullum et al. 2004). The most likely route of exposure for populations living in the vicinity of Army ammunition plants is ingestion of contaminated drinking water or agricultural crops that have been irrigated with contaminated water (Harvey et al. 1991, 1997; Simini and Checkai 1996). Dermal contact with soil containing RDX and inhalation of contaminated particulate matter produced during incineration of RDX-containing waste material are also possible routes of exposure. However, since no monitoring data were located regarding levels of RDX in outdoor air, the extent of exposure by this route is not known. Dermal contact with contaminated soil is also a possible

route of exposure. However, since no absorption data following dermal exposure to RDX were located, the extent of exposure by this route is also not known.

Occupational exposure to RDX can occur when workers handle RDX in explosive plants (Hathaway and Buck 1977; Kaplan et al. 1965; Testud et al. 1996b). Inhalation exposure of workers to RDX has occurred as a result of release of dust into the workroom air, principally during dumping of dried RDX powder, screening and blending, and clean-up of spilled material (Kaplan et al. 1965; Testud et al. 1996b). Exposure to RDX can also occur through dermal contact during manufacture, handling, and clean-up of RDX (Kaplan et al. 1965). RDX was detected at a concentration of 0.052 mg/$m^3$ (0.47 ppm) in the particulate fraction of one indoor air sample taken from the incorporation area of Holston Army Ammunition Plants in Tennessee in 1986 (Bishop et al. 1988). Based on the observed concentration, the potential for exposure to RDX is considered to be very low.

According to the NOES (1981–1983), the estimated number of workers potentially exposed to RDX in the United States was 488 (NIOSH 1990).

## 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).

Children can be exposed to RDX by inhalation, oral, or dermal contact with the chemical or by any combination of these routes. Children residing in areas around Army ammunition plants where RDX is manufactured, converted to munitions, or released through the demilitarization of antiquated munitions may be exposed to RDX (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1984a,

1984f). The primary route of exposure is ingestion of contaminated drinking water. Inhalation exposure may result from breathing contaminated particulate matter produced during incineration of RDX-containing waste material. Dermal contact with contaminated soil is also a possible route of exposure. Children playing in contaminated water or soil may also be exposed via ingestion.

## 6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Exposure of workers can occur via the inhalation, oral, or dermal routes, or by any combination of these routes. Workers involved in the production and use of RDX at Army ammunition plants constitute a group at risk because of the potential for occupational exposure. Persons living near Army ammunition plants or hazardous waste sites may have a higher risk of exposure to RDX resulting from inhalation of dusts or fumes, ingestion of contaminated drinking water, or contact with contaminated soil (Hundal et al. 1997; Pennington and Brannon 2002; Testud et al. 1996b). Military personnel may also be exposed to high levels from the use of explosives that contain RDX. Individuals employed in demilitarization of nuclear, biological, and chemical weapons as per international treaty agreements may be exposed to high levels of RDX, as disassembly of these missiles involves disassembly of RDX-containing bursters and detonators.

## 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 RDX is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to ensure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of RDX.

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

### 6.8.1 Identification of Data Needs

**Physical and Chemical Properties.** The physical and chemical properties of RDX are sufficiently characterized to permit estimation of its environmental fate (Akhavan 2004; Budavari and O'Neil 1989; McKone and Layton 1986; U.S. Army 1986e, 1987a).

**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 2006, became available in February of 2008. This database is updated yearly and should provide a list of industrial production facilities and emissions.

RDX is not produced commercially in the United States. Production in the United States is limited to Holston Army Ammunition Plants in Kingsport, Tennessee (SRI 2009),. Current import/export data for RDX are not available. RDX is primarily used as a high explosive (Boileau et al. 2009; HSDB 2009; Lewis 2007; Budavari and O'Neil 1989; Turley and Brewster 1987). RDX is primarily found in water, groundwater, and soil around Army ammunition plants (Agency for Toxic Substances and Disease Registry 1989a, 1989b, 1989c; Bart et al. 1997; Bishop et al. 1988; Dacre 1994; Simini et al. 1995; Spalding and Fulton 1988; U.S. Army 1988). Data on the most commonly used disposal methods are sufficient (Hoffsommer and Rosen 1972; U.S. Army 1986a, 1986c); however, additional data on the amounts of RDX being disposed of and on alternative disposal methods would be useful. RDX wastes produced in manufacturing and processing are classified as hazardous wastes and are subject to EPA regulations (EPA 1990a).

**Environmental Fate.** RDX released to the environment partitions into air, water, and soil (Eisenreich et al. 1981; Lyman et al. 1982; U.S. Army 1980a, 1983b, 1987a). RDX is transported in soil, surface water, and groundwater (Swann et al. 1983; U.S. Army 1980c, 1983b, 1985a, 1986e, 1987a). Volatilization is expected to be a slow transport process (Lyman et al. 1982). RDX is expected to exist as a particulate in the atmosphere. No data were located in the literature regarding atmospheric transport of RDX. Experimental data are needed regarding photolysis of RDX in the atmosphere. Photolysis is the primary mechanism of RDX degradation in water (half-life of 9–13 hours) (U.S. Army 1980a, 1986e). Biodegradation of RDX occurs in water and soil, principally under anaerobic conditions (Funk et al. 1993; McCormick et al. 1981; Osmon and Klausmeier 1973; Pennington and Brannon 2002; U.S. Army 1984f, 1985a). Biodegradation half-life data for RDX and its breakdown products in water and soil are

needed. This information will be helpful in better identifying the most important pathways of human exposure to RDX.

**Bioavailability from Environmental Media.** Absorption data regarding dermal exposure in humans are not available. Very limited data indicate that RDX is absorbed following inhalation exposure (Kaplan et al. 1965; Testud et al. 1996b). RDX is absorbed through the gastrointestinal system following ingestion of the compound (Hollander and Colbach 1969; Ketel and Hughes 1972; Merrill 1968; Stone et al. 1969). The oral and dermal routes of exposure may be of concern to humans because of the potential for RDX to contaminate drinking water and soil. More information regarding all absorption routes, particularly on the absorption of RDX following ingestion of contaminated drinking water and soil or plants grown in contaminated environments, is needed to better characterize the bioavailability of RDX.

**Food Chain Bioaccumulation.** Based on a low log $K_{ow}$ and low experimental BCF values of 1.2–5.9, RDX has a low bioconcentration potential in aquatic organisms (PHYSPROP 2009; U.S. Army 1984a). No data were located regarding bioconcentration potential in animals. Data are needed regarding bioconcentration/biomagnification potential in terrestrial food chains.

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

RDX has been detected in surface water, groundwater, and soil at Army ammunition plants and current and former military installations (Agency for Toxic Substances and Disease Registry 1989a, 1989b, 1989c; Bart et al. 1997; Simini et al. 1995; Spalding and Fulton 1988). Data are needed regarding levels of RDX in ambient air and occupational air. No data were located regarding human intake estimates for each media. Reliable monitoring data are needed for levels of RDX in contaminated media at hazardous waste sites. The information on RDX levels in the environment and the resulting body burden of RDX can be used to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

**Exposure Levels in Humans.** RDX has been detected in surface water, groundwater, and soil at Army ammunition plants (Agency for Toxic Substances and Disease Registry 1989a, 1989b, 1989c; Bart

et al. 1997; Simini et al. 1995; Spalding and Fulton 1988). Data are needed regarding levels of RDX in ambient air and occupational air. No data were located regarding human intake estimates for each media. Reliable monitoring data are needed for levels of RDX in contaminated media at hazardous waste sites. The information on RDX levels in the environment and the resulting body burden of RDX can be used to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

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

**Exposures of Children.** RDX has been detected in surface water, groundwater, and soil at Army ammunition plants (Agency for Toxic Substances and Disease Registry 1989a, 1989b, 1989c; Bart et al. 1997; Simini et al. 1995; Spalding and Fulton 1988). Data are needed regarding levels of RDX in ambient air. No data were located regarding human intake estimates for each media. Reliable monitoring data are needed for levels of RDX in contaminated media at hazardous waste sites as well as potential uptake by children through ingestion of drinking water and contaminated crops, and accidental ingestion of contaminated soils. Dermal contact is also a concern for children playing in or near contaminated areas. The information on RDX levels in the environment and the resulting body burden of RDX can be used to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

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 RDX 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.

### 6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2009) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-1.

## Table 6-1. Ongoing Studies on RDX

| Investigator | Affiliation | Research description | Sponsor |
|---|---|---|---|
| Chu, K | Texas Engineering Experiment Station | Molecular probing and identification of active RDX-utilizing microorganisms | NSF |
| Schnoor, JL | University of Iowa | Involvement of an endosymbiotic *Methylobacterium sp.* in the biodegradation of explosive RDX and HMX inside poplar tree (*Populus deltoids x Populus nigra*) | NSF |

NSF = National Science Foundation

Source: FEDRIP 2009

# 7. ANALYTICAL METHODS

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

The most common procedures for the analytical separation of RDX in biological and environmental materials are high-performance liquid chromatography (HPLC) and gas chromatography (GC). These methods have been paired with several types of detectors, including thermal energy analyzer (TEA), electrochemical detector (ED), electron capture detector (ECD), and ultraviolet (UV). The TEA is very selective for nitroso compounds and when paired with either HPLC or GC, gives excellent selectivity, recovery, and precision and high sensitivity (Fine et al. 1984; Lafleur and Morriseau 1980). The limited reports of analysis of materials using HPLC and ED indicate detection limits in the low ppb range and good reliability (Krull et al. 1984; Lloyd 1983). GC coupled with ECD appears to have good sensitivity (low ppb), accuracy, and precision (Bishop et al. 1981, 1988). UV detection has also been used with HPLC separation, but few data are available for comparison with other methods (Burrows and Brueggemann 1985; Strobel and Tontarski 1983). The data suggest that this method has very good accuracy and precision; however, the selectivity may not be as good as that obtained with other detectors. Methods based on mass spectrometry (MS) with sensitivity in the sub-ppb range have been described, but specific information on their reliability is limited (St. John et al. 1975; Tanner et al. 1983). MS is generally accepted to be highly selective. Sample preparation for RDX analytical methods is relatively simple, consisting of collection of the sample from air, water, soil, tissue, fluid, residue, or waste followed by homogenization if necessary, one or two extraction/clean-up steps, and concentration of the sample.

## 7.1 BIOLOGICAL MATERIALS

Analytical methods specifically used for the determination of RDX in biological fluids and tissues are limited. Methods were located that discussed the analysis of RDX in blood, tissues, urine, and hand swabs. The separation methods employed included high-performance liquid chromatography (HPLC) or gas chromatography (GC). These were combined with detection by thermal energy analyzer (TEA), ultraviolet (UV), electrochemical detector (ED), or electron capture detector (ECD). Both HPLC and high-resolution gas chromatography (HRGC) can rapidly separate RDX from other explosives, but HPLC has the advantage of being run at ambient temperature, which helps prevent breakdown of the analyte. Pertinent data on these methods are presented in Table 7-1.

Detection of RDX in human and animal plasma as well as human urine and cerebrospinal fluid has been accomplished by HPLC/TEA and HPLC/UV (U.S. Army 1981a; Fine et al. 1984; Turley and Brewster 1987). While both methods provide relatively rapid sample turn-around times, HPLC/TEA is the most sensitive and selective of the two, and requires little sample preparation (Fine et al. 1984). The older HPLC/UV method (U.S. Army 1981a) had the problem of coelution of a plasma component with the RDX peak. This was eradicated by clean-up on a C18 bonded-phase extraction column (Turley and Brewster 1987; Woody et al. 1986), but the sensitivity of HPLC/UV was still several orders of magnitude less (limit of detection in low ppb) than that of HPLC/TEA (limit of detection in low ppt). Reported recoveries ranged from 87.7 to 101% (Turley and Brewster 1987; U.S. Army 1981a; Woody et al. 1986). Precision was comparable and ranged from 0.65 to 10% coefficient of variation (CV).

A method of analyzing feces for RDX was located (Woody et al. 1986). This method used HPLC/UV and required extraction of the sample with acetonitrile and sonication. The limit of detection was not reported, although based on the data presented, it was assumed to be in the low ppb range.

One method was located for analysis of tissue samples. The method used HPLC/UV to analyze bovine kidney, muscle/fat, and liver samples for RDX, but it could be used to analyze human tissues (U.S. Army 1981a). Optimal sample preparation methods varied slightly for the different tissues, as did detection limits and precision. In general, the detection limit was in the low ppb and recovery was high (in the range of 87.7–102.9). Precision ranged from 7 to 16% CV. The primary issue with analysis of tissue using this method is the variation in selectivity. Minor differences in sample extraction and contamination from unknown sources can create interferences that drastically affect interpretation of results and may also adversely affect the sensitivity.

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Plasma | Extract with methylene chloride and pentane; filter; concentrate | HPLC/TEA | 100 ng/L | No data | Fine et al. 1984 |
| Plasma | Add NaCl/acetic acid solution to sample; extract with toluene; add water; evaporate organic phase; combine aqueous phase with acetonitrile-containing internal standard; filter | HPLC/UV | 146 µg/L | 87.7 (spike levels 0–2,000 ng/g; SD±19–188 ng; CV 7–19) | U.S. Army 1981a |
| Serum and urine | Mix sample with internal standard; clean up on $C_{18}$-bonded-phase extraction column, eluting with methanol; concentrate | HPLC/UV | 100 µg/L | 90±2.0–101±1.1 (1–10 mg/L in serum); 98±1.6–101±1.3 (1–10 mg/L in urine) | Turley and Brewster 1987 |
| Kidney | Add NaCl/acetic acid solution to sample; extract with toluene; add water; evaporate organic phase; combine aqueous phase with acetonitrile-containing internal standard; filter | HPLC/UV | 95 ng/g | 99.5 (spike levels 0–2,000 ng/g; SD±12–58; CV 2–11) | U.S. Army 1981a |
| Muscle/fat | Homogenize sample; extract with acetonitrile; concentrate; add internal standard and purified water; filter | HPLC/UV | 62 ng/g | 102.9 (spike levels 0–2,000 ng/g; SD±2.2–86; CV 3.9–14) | U.S. Army 1981a |
| Liver | Homogenize sample; add NaCl/acetic acid solution; evaporate; redissolve in acetonitrile-containing internal standard; filter | HPLC/UV | 150 ng/g | 87.7 (spike levels 0–1,000 ng/g; SD±18–69; CV 7–22) | U.S. Army 1981a |
| Hand swabs | Wipe hand with swab soaked in acetone; squeeze out acetone and concentrate | HPLC/TEA; HRGC/TEA | 10 pg/inj | No data | Fine et al. 1984 |

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Hand swabs | Wipe hand with swab soaked in ether; extract with ether; centrifuge to remove debris; decant supernatant and evaporate; redissolve in pentane; clean up on Amberlite XAD-7 beads, eluting with ethyl acetate; evaporate; redissolve in pentane and repeat Amberlite XAD-7 clean-up | GC/ECD | 50 ng/ swab (1.7 ng/inj) | 47 (at 200 ng/swab) | Douse 1982 |
| | | TLC | 20 ng/ swab | No data | |
| Hand swabs, standards | Wipe hand with dry swab; extract with methanol/ potassium phosphate; directly inject standards | HPLC/PMDE | 8 pg/inj (standards) | No data | Lloyd 1983 |

CV = coefficient of variation; ECD = electron capture detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; inj = injection; PMDE = pendant mercury drop electrode; TEA = thermal energy analyzer; TLC = thin layer chromatography; UV = ultraviolet

The only other methods for biological matrices located were for analysis of hand swabs. These are of primary importance in forensics, but they could also be used to determine if dermal exposure of workers has occurred. Methods that have been used for the determination of trace amounts of RDX on hands include HPLC with TEA or electrochemical detection and HRGC with TEA or ECD (Douse 1982; Fine et al. 1984; Lloyd 1983). Thin-layer chromatography has also been tested, but because of the large amounts of sample that are required for the analysis, it is useful only as a screening test for high concentration samples (Douse 1982). Separation of the sample by HPLC and HRGC are comparable, but reported recovery for HRGC is low (Douse 1982). This is likely because of decomposition of the sample, but the data are not available to adequately compare the recovery of the two methods. The nature of the detector seems to be the most important factor in determining which of the reported methods is most useful for the analysis of RDX in hand-swab extracts. ECD appears to be less sensitive (ng amounts) than either electrochemical detection using the pendant mercury drop electrode (PMDE) or TEA (pg amounts). In addition, in the method reported, clean-up was required to prevent matrix interference (Douse 1982). For both the PMDE and TEA methods, clean-up of the sample was not required, and both methods were rapid, selective, and of high precision (Fine et al. 1984; Lloyd 1983).

## 7.2 ENVIRONMENTAL SAMPLES

A large variety of methods have been described for the detection of RDX in environmental samples. These primarily include HRGC combined with ECD, TEA, mass spectrometry (MS), or flame ionization detection (FID); HPLC combined with UV, TEA, MS, photoconductivity (PD), or electrochemical detection; automated multiple development high performance thin-layer chromatography (HPTLC-AMD); liquid chromatography (LC) with thermospray (TSP) and MS; and several stand-alone MS techniques. Other methods have also been proposed, including fluorescent quenching; supercritical fluid chromatography (SFC) with UV. Table 7-2 is a summary of several representative methods for determining RDX in various environmental media.

Several methods for determining RDX in air have been investigated. Based on the limited data available, the two most common methods are GC/ECD and MS. The data reported are not sufficient to make comparisons of sensitivity and reliability between the methods. GC/ECD, however, appears to have good sensitivity (low ppb), accuracy, and precision (Bishop et al. 1981, 1988). An alternate method based on spectrophotometry also provided similar results for accuracy and precision (±12.4% CV) and had a detection limit of the same order of magnitude as that reported using GC/ECD (Eminger and Vejrostova

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air | Collect sample on Tenax-plus-filter tubes; desorb with acetonitrile | HRGC/ECD | 17 µg/$m^3$ | No data | Bishop et al. 1988 |
| Air | Collect sample on Tenax-GC; desorb with acetonitrile | HRGC/ECD | No data | 93–102; 98±4.4 average (6–120 µg test level) | Bishop et al. 1981 |
| Air | Collect sample on glass-fiber filter; extract with ethyl acetate | GC/FID | 0.5 mg/$m^3$ | No data (precision ±15%) | U.S. Army 1975 |
| Air | Collect sample in sampling tube of glass-microfibers and silica gel; transfer to $H_2SO_4$ solution and react with dihydroxynapthalene-disulfonic acid and water; dilute with water | Spectro-photometry | 40 µg/$m^3$ | 95.7–97.3 | Eminger and Vefrostova 1984 |
| Air | Incorporate sample into bulb containing isotopically-labeled RDX; extract with benzene; transfer to capillary tube and evaporate | IDMS | Sub-ppb | No data | St. John et al. 1975 |
| Air | Inject sample directly into instrument | APCI/MS/MS | Sub-ppb | No data | Tanner et al. 1983 |
| Waste water effluents | Add internal standard to sample; elute from reverse-phase column with methanol/water | HPLC/UV | 0.2 mg/L | 100–102 (measured at 0.67 mg/L; RSD 0.36–9.48% measured at 0.27–2.66 ppm) | U.S. Army 1983c |
| Groundwater, waste water effluents | Dilute sample with methanol/acetonitrile; filter; elute from reverse-phase column with water/acetonitrile/ methanol | HPLC/UV | 22 µg/L | 101 | Jenkins et al. 1986; U.S. Army 1985c |

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Groundwater | Collect sample on Hayesep R solid sorbent cartridge; elute with acetone; concentrate; add internal standards; dilute with methanol/ water | HPLC/UV/ UV/PD | 5–7.5 µg/L | 104–121 | U.S. Army 1989a |
| Surface water, well water | Collect sample on Porapak resin; rinse sorbent with distilled water and elute with acetone; concentrate; add ethanol; concentrate; add methanol/water | HPLC/ED | ≈1 µg/L | 57–63 | Maskarinec et al. 1984 |
| Water | Collect sample on XAD-4 resin; elute with ethyl acetate; concentrate | HRGC/ECD | <0.1 µg/L | 97±5 (spike level 4 µg/L) | Richard and Junk 1986 |
| Water | Liquid/liquid extraction using dichloromethane | HPTLC-AMD | 10 ng | No data (RSD 1.6–5.9% for 20–130 ng in solution) | Steuckart et al. 1994 |
| Groundwater, drinking water | Extract sample with isoamyl acetate | HRGC/ECD | 0.3 µg/L | 56–84 (spike level 0.15–3.0 µg/L; RSD 9.3–19) | Hable et al. 1991 |
| Sea water | Add internal standard to sample; extract with benzene; evaporate; redissolve in benzene | GC/ECD | 5 ng/L | 70±10 (at 103–1,400 ng/L) | Hoffsommer and Rosen 1972 |
| Water | Evaporate sample; redissolve in acetone; filter; concentrate | HRGC/ECD | 60 ng/L | 85 | Haas et al. 1990 |
| Water | Inject sample directly into instrument | MS (CI) | 40 mg/L | No data | Yinon and Laschever 1982 |
| Groundwater | Add sample to cyclo-hexanone/ pyrenebutyric acid/ cellulose triacetate/ isodecyl diphenyl-phosphate membrane in cuvette | Fluorescense quenching | ≈10 mg/L | No data | Jian and Seitz 1990 |

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Soil | Air-dry, grind, and sieve sample; extract with acetonitrile in ultrasonic bath; add $CaCl_2$; filter; elute from reverse-phase column with water/methanol | HPLC/UV | 0.74 µg/g | 84–112 (multilaboratory determination) | Bauer et al. 1990; Jenkins and Grant 1987; Jenkins et al. 1989; U.S. Army 1987b (interim AOAC method) |
| Soil | Adjust sample moisture to 20–30%; homogenize and sieve; extract with acetonitrile and sonication; centrifuge and filter; elute from reverse-phase column with methanol/water | HPLC/UV | 0.6 µg/g | 103.7 (spike level 0.5–200 µg/g; CV 0.098) | Bongiovanni et al. 1984 |
| Soil | Air-dry sample; extract with acetonitrile; filter; evaporate; redissolve in acetonitrile; elute from reverse-phase column with acetonitrile/water | HPLC/UV | 0.005 µg/g | No data | Lyter 1983 |
| Soil | Homogenize sample; extract with acetone; filter | HRGC/ECD | 75 ng/g | 95 | Haas et al. 1990 |
| Soil | Homogenize sample; extract with acetone; evaporate; react with diphenylamine/$H_2SO_4$ | Spectro-photometry | 5 mg/L | No data | Haas et al. 1990 |
| Soil | Grind sample; extract with acetone in ultra-sonic bath, centrifuge, add toluene, and dry; remove humic substances with calcium chloride or elution with ethyl acetate/petroleum ether over biobeads | HPTLC-AMD | 10 ng | No data (RSD 1.6–5.9% for 20–130 ng in solution) | Steuckart et al. 1994 |
| Soil | Extract of soil sample and enzyme conjugate reagent added to immobilized RDX antibody; D TECH™ RDX test kit required | Immunoassay | 5 µg/g | 53–114 (spike level 0.53–6.82 mg/g; SD 0.12–1.21; CV 5–46%) | EPA 1996 |

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Soil | Soil samples extracted with acetone; extract passed through ion exchange resin; extract acidified and mixed with zinc dust, color developed using a NitriVer 3 powder pillow | Colorimetric screening using spectro-photometry | 1 µg/g | 60–140 | EPA 2007 |
| Agricultural crops (tomato) | Acid hydrolysis; extract with diethyl ether | HPLC | 5 ng/g (laboratory-grown); 17 ng/g (field-grown) | 90±4 (laboratory-grown; spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (soybean) | Acid hydrolysis; extract with diethyl ether | HPLC | 50 ng/g (laboratory-grown) | 70±3 (spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (corn) | Acid hydrolysis; extract with diethyl ether | HPLC | 51 ng/g (laboratory-grown, stover); 13 ng/g (field-grown, kernel) | 75±18 (laboratory-grown; spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (bush bean) | Acid hydrolysis; extract with diethyl ether | HPLC | 8 ng/g (laboratory-grown) | 68±11 (spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (radish) | Acid hydrolysis; extract with diethyl ether | HPLC | 3.2 ng/g (laboratory-grown) | 103±38 (spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (alfalfa) | Acid hydrolysis; extract with diethyl ether | HPLC | 15 ng/g (laboratory-grown) | 76±3 (spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (lettuce) | Acid hydrolysis; extract with diethyl ether | HPLC | 7 ng/g (laboratory-grown) | 71±9 (spike level 5 µg/g dry mass) | Harvey et al. 1997 |
| Agricultural crops (hot pepper) | Acid hydrolysis; extract with diethyl ether | HPLC | 28 ng/g (field-grown) | No data | Harvey et al. 1997 |
| Agricultural crops (carrot) | Acid hydrolysis; extract with diethyl ether | HPLC | 39 ng/g (field-grown) | No data | Harvey et al. 1997 |
| Agricultural crops (green pepper) | Acid hydrolysis; extract with diethyl ether | HPLC | 20 ng/g (field-grown) | No data | Harvey et al. 1997 |

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

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Agricultural crops (grapes) | Acid hydrolysis; extract with diethyl ether | HPLC | 18 ng/g (field-grown) | No data | Harvey et al. 1997 |
| Explosive preparations | Elute from HPLC column with isooctane/ethanol | HPLC/TEA | No data | 98–102 | Lafleur and Morriseau 1980 |
| Explosives, explosion debris | Dissolve sample in acetone; dilute in methanol | HPLC/TEA; HRGC/TEA | Low pg | No data | Fine et al. 1984 |
| Explosives | Extract sample with acetone; elute from HPLC column with methanol/potassium phosphate | HPLC/EC (PMDE) | 8 pg/g | No data (CV 0.8% of 1 ng replicates) | Lloyd 1983 |
| Explosion debris | Extract sample in acetone; clean up on cyclohexyl column; eluting with methylene chloride/hexane; clean up on cyanopropyl column; elute with acetonitrile/water | HPLC/UV | No data | 99 | Strobel and Tontarski 1983 |
| Munitions products | Dissolve sample in acetonitrile; add water; elute from reverse-phase column with methanol/water | HPLC/UV | No data | No data | Burrows and Brueggemann 1985 |
| Explosives | Extract with acetone; evaporate; redissolve in dichloroethane; elute from HPLC column with dichloroethane/hexane | HPLC/MS (CI) | ≈1 ng | No data | Vouros et al. 1977 |
| Explosives, explosive residues | Dissolve in acetone or methanol; elute from HPLC column with methanol/ammonium acetate | HPLC/TSP/MS | Low pg | No data | Berberich et al. 1988 |

AMD = automated multiple development; APCI = atmospheric pressure chemical ionization; AOAC = Association of Official Analytical Chemists; $CaCl_2$ = calcium chloride; CI = chemical ionization; CV = coefficient of variation; EC = electrochemical detection; ECD = electron capture detection; ED = electrochemical detection; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HPTLC = high performance thin-layer chromatography; HRGC = high-resolution gas chromatography; $H_2SO_4$ = sulfuric acid; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; PD = photoconductivity detection; PMDE = pendant mercury drop electrode; RSD = relative standard deviation; SD = standard deviation; TEA = thermal energy analyzer; TSP = thermospray; UV = ultraviolet detection

1984). MS methods with sensitivity in the sub-ppb range have been described, but specific information on their reliability is limited. MS is generally accepted to be highly selective. Of the two MS methods described, isotope dilution MS (IDMS) (St. John et al. 1975) and MS/MS with atmospheric pressure chemical ionization (APCI) (Tanner et al. 1983), the latter (APCI/MS/MS) is the most rapid and simple to perform because the sample of air containing RDX vapors is directly injected into the instrument. The high sensitivity and selectivity of MS/MS allow the air sample to be injected without prior treatment or concentration. However, the method as presented appears to be primarily useful as a screening technique to determine if more rigorous quantitative analysis is required. IDMS requires some sample preparation in order to incorporate the known amount of labeled analyte in the sample containing the unknown amount of RDX. IDMS has been used to measure the vapor pressure of RDX, which is in the sub-ppb range.

The primary analytical methods for determining RDX in water are HPLC/UV and GC/ECD. These methods have been used to determine the chemical in waste-water effluents, groundwater, well water, drinking water, and seawater. The critical step in the analysis of RDX by HPLC/UV is separation of the sample on a reverse-phase column, which provides good selectivity without risk of thermal breakdown of the analyte (Jenkins et al. 1986; U.S. Army 1983c, 1985c). The method is simple, quick, and reproducible. Sensitivity is in the low- to mid-ppb range, with good recovery and excellent precision (2–7.6% CV). The use of HPLC in combination with photodiode-array detection improves the reliability of peak identification (Emmrich et al. 1993). The HPLC-photodiode-array detection method can provide a detection limit of 0.09 ppb for RDX in aqueous samples concentrated 1,000-fold by liquid-liquid extraction or by solid phase extraction (C-18) (Levsen et al. 1993). The extraction efficiency of RDX from water to acetonitrile can be improved by using salting out agents (U.S. Army 1991). The sensitivity and selectivity of RDX detection was improved by combining a solid sorbent cartridge to concentrate RDX from water and HPLC-tandem ultraviolet and photoconductivity detection (HPLC/UV/PD) (U.S. Army 1989a). The system consisted of a UV absorbance detector set to 254 nm and a photoconductivity detector equipped with a zinc photoionization source. The serial use of the two detectors effectively differentiated RDX from other explosives and from contaminants in the solid sorbent cartridge. In addition, the sensitivity was improved by a factor of about 3. To prevent negative baseline drift and random spikes in the PD, only highly purified water must be used, and the effluent must be exhaustively degassed (U.S. Army 1989a). Automated multiple development high performance thin-layer chromatography (HPTLC-AMD) has also been used to analyze water samples. Liquid-liquid extraction using dichloromethane was used to prepare the samples. A detection limit of 10 ng was obtained (Steuckart et al. 1994).

For analysis by GC/ECD, water samples may be solvent-extracted (Belkin et al. 1985; Haas et al. 1990; Hable et al. 1991; Hoffsommer and Rosen 1972) or collected on a solid sorbent (Richard and Junk 1986). Solvent extraction is most commonly used, but solid sorbent collection has the advantages of being faster and cheaper than solvent extraction (Richard and Junk 1986). Sensitivity for the GC/ECD methods ranges from low to mid ppt, and the recovery and precision are acceptable. Use of the solid sorbent improved recovery and precision compared to solvent-extraction methods (Richard and Junk 1986). Substitution of ED, using a gold-mercury electrode, improved selectivity compared to ECD detection. Sensitivity was not as good, but it remained within an order of magnitude of that found with GC/ECD (Maskarinec et al. 1984). Recovery and precision were comparable. A more recent study indicated that GC/ECD is not useful in the determination of RDX in water samples, as RDX may undergo thermal degradation (Steuckart et al. 1994).

Other methods that have been used to determine RDX in water are MS, fluorescence quenching, COD, and total organic carbon (TOC) (Jian and Seitz 1990; Roth and Murphy 1978; Yinon and Laschever 1982). COD and TOC (Roth and Murphy 1978) are well-established standard methods for determining organic pollution in water, but they are not selective for RDX. MS with chemical ionization (CI) permits direct injection of the water sample into the analytical instrument, but the sensitivity is substantially less than with the HPLC/UV and GC/ECD methods (Yinon and Laschever 1982). Fluorescence quenching also lacks sensitivity, and the method is still under development. However, it does permit *in situ* measurement of samples, and further improvements in the technology may make it a desirable field method (Jian and Seitz 1990). Continuous flow immunosensor (CFI) has been found to produce results comparable to HPLC in detecting RDX in groundwater samples (Bart et al. 1997). CFI utilizes a small column of plastic beads containing immobilized antibodies with the explosive and a fluorescent dye-labeled explosive analog. When the explosive present in the sample displaces the dye-labeled analog in the column, the explosive is detected with a detection limit of approximately 20 ppb (Bart et al. 1997).

The methods that were located for detection of RDX in soil are based primarily on HPLC/UV analysis (Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins and Grant 1987; Jenkins et al. 1989; Lyter 1983; U.S. Army 1987b). All of the methods involve extraction of the sample with acetonitrile, separation using a reverse-phase column, and in most cases, elution with acetonitrile/water. Sensitivity for these methods is in the sub- to low-ppm range with good recovery (84–112%) and precision (2.3–24% CV). A variation of the method involves the soil sample being extracted with acetonitrile in an ultrasonic bath (Jenkins et al. 1989; Steuckart et al. 1994). Soil samples can be ground into mortar and extracted with acetone in an

ultrasonic bath maintained at ambient temperature, centrifuged, added to toluene, and dried over anhydrous sodium sulfate. Steuckart et al. (1994) removed humic substances with either a calcium chloride solution or elution with ethyl acetate/petroleum ether over biobeads. The samples were analyzed by HPTLC-AMD with a detection limit of 10 ng (Steuckart et al. 1994).

Other analytical methods are based on GC/ECD and spectrophotometry (Haas et al. 1990). In both of these methods, the samples were extracted with acetone. The detection limit for spectrophotometric determination of RDX in soil was in the low-ppm range, while the detection limit for GC/ECD was in the mid-ppb range. No information on accuracy and precision were given for the spectrophotometric method; however, the accuracy of GC/ECD was comparable to HPLC/UV.

Methods are available for identification of RDX in agricultural crops. Harvey et al. (1997) utilized HPLC to determine RDX concentrations. The samples underwent acid hydrolysis with hydrochloric acid and extraction with diethyl ether prior to analysis by HPLC. The detection limits and percent recoveries for a variety of crops are listed in Table 7-2 (Harvey et al. 1997). Larson et al. (1999b) used an 18-hour cooled sonication extraction technique using acetonitrile to extract RDX from plant tissues that had been exposed to contaminated irritation water. The samples were then analyzed with HPLC/UV.

Several methods have been used to detect and measure RDX in explosive materials and debris from explosions. The most common separation procedure is HPLC, but HRGC has also been used. These methods have been paired with several types of detectors, including TEA, MS, electrochemical detection, and UV. The TEA is very selective for nitroso compounds and when paired with either HPLC or HRGC, gives excellent selectivity, recovery, and precision and high sensitivity (Fine et al. 1984; Lafleur and Morriseau 1980). GC/MS has been used for confirmation of RDX in samples of explosive materials (Burrows and Brueggemann 1985), and HPLC/MS and MS/MS have been investigated as screening methods for explosives (McLucky et al. 1985; Vouros et al. 1977). A sophisticated method linking HPLC, thermospray (TSP), and MS or MS/MS (with both positive and negative chemical ionization) has also been proposed as an extremely sensitive (low pg range) and selective method for detecting RDX in explosive residues (Berberich et al. 1988; Verweij et al. 1993). However, there is no evidence that any MS-based method is currently used to quantitatively measure RDX in explosives or explosion debris. A relatively new method being investigated uses supercritical fluid extraction chromatography (SFC) to separate RDX from other analytes and contaminants followed by detection by UV/FID (Griest et al. 1989). The method is slower but more selective than HPLC/UV. The precision for standard solutions

was excellent. However, more work is needed to improve the mobile phase and column packing material before samples in complex matrices can be analyzed.

## 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 RDX is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to ensure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of RDX.

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.

***Exposure.*** Few methods exist for monitoring exposure to RDX. Methods have been reported for detection of the analyte in plasma (Fine et al. 1984; Turley and Brewster 1987; U.S. Army 1981a; Woody et al. 1986), urine (Turley and Brewster 1987; Woody et al. 1986), cerebrospinal fluid (Woody et al. 1986), feces (Woody et al. 1986), and tissues (U.S. Army 1981a), as well as on hands (Douse 1982; Fine et al. 1984; Lloyd 1983). The available methods can detect levels in urine and plasma from exposure to concentrations below those that would be encountered in most manufacturing situations. In general, these methods are reliable and accurate; however, the development of the LC-MS methodology could be useful as a definitive method to validate the specificity of the HPLC methods. The data are insufficient to permit correlation of RDX levels in the urine or blood with exposure levels.

***Effect.*** There are no known sensitive biomarkers of effect for RDX. Therefore, no methods recommendations can be made for this chemical.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media**. Methods exist to detect and quantify RDX in air (Bishop et al. 1988; Eminger and Vejrostova 1984; St. John et al. 1975; Tanner et al. 1983; U.S. Army 1974), water (Haas et al. 1990; Hable et al. 1991; Jian and Seitz 1990; Maskarinec et al. 1984; Richard and Junk 1986; Steuckart et al. 1994; U.S. Army 1983c, 1985c, 1989a; Yinon and Laschever 1982), soil (Bongiovanni et al. 1984; Haas et al. 1990; Steuckart et al. 1994; U.S. Army 1987b), agricultural crops (Harvey et al. 1997; Larson et al. 1999b), explosive materials (Burrows and Brueggemann 1985; Fine et al. 1984; Lafleur and Morriseau 1980; Lloyd 1983), and debris from explosions (Fine et al. 1984; Strobel and Tontarski 1983). These methods are relatively sensitive and reliable and can be used to detect levels of the compound in the environment that cause known adverse health effects. There are some problems involving reduced sensitivity and selectivity with all of the commonly used methods. Several proposed improvements in current methods, such as combining various analytical methods to increase selectivity, sensitivity, reliability, and/or accuracy (Berberich et al. 1988; Krull et al. 1984; U.S. Army 1989a), and investigations of new methods (Griest et al. 1989; Jian and Seitz 1990) will be useful in forensics and in monitoring environmental contamination from manufacture and disposal of RDX.

### 7.3.2 Ongoing Studies

The information in Table 7-3 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2009).

## Table 7-3. Ongoing Studies on RDX

| Investigator | Affiliation | Research description | Sponsor |
|---|---|---|---|
| Ram, M | Triton Systems, Inc. Auburn University | *In situ* near real-time detection of RDX in soil | U.S. Army |
| Li, J | University of Florida | Enhanced quadrupole resonance technology for explosive detection | NSF |
| Indacochea, JE | University of Illinois at Chicago | Development of a nanostructured-based sensor system for reliable detection of improvised explosive devices | NSF |
| Scherer, JJ | NovaWave Technologies | Ultrasensitive, real-time explosives sensor | NSF |

NSF = National Science Foundation

Sources: DOD 2009; EPA 2008b; FEDRIP 2009

# 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.

The international and national regulations, advisories, and guidelines regarding RDX in air, water, and other media are summarized in Table 8-1.

ATSDR has derived an acute-duration oral MRL of 0.2 mg/kg/day based on a NOAEL of 8.5 mg/kg/day for neurotoxicity in rats administered RDX via gavage 7 days/week for 14 days (U.S. Army 2006b). Using a PBPK model, an internal dose metric (peak brain concentration) was simulated and a HED of 6.4547 mg/kg/day was estimated. An uncertainty factor of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability) was applied to the $NOAEL_{HED}$.

ATSDR has derived an intermediate-duration oral MRL of 0.1 mg/kg/day based on a $BMDL_{10}$ for neurological effects in rats administered RDX via gavage 7 days/week for 90 days (U.S. Army 2006b). The $BMDL_{10}$ was estimated using an internal dose metric to simulate peak brain concentration; a HED of the $BMDL_{10}$ was estimated using a PBPK model. The $BMDL_{HED}$ of 4.1308 mg/kg/day was divided by an uncertainty factor of 30 (3 for animal to human extrapolation with dosimetric adjustments and 10 for human variability).

ATSDR has derived a chronic-duration oral MRL of 0.1 mg/kg/day based on a NOAEL of 8 mg/kg/day for neurotoxicity in rats exposed to dietary RDX for 2 years (U.S. Army 1983a). Using a PBPK model, an internal dose metric (peak brain concentration) was simulated and a HED of 4.223 mg/kg/day was estimated. An uncertainty factor of 30 (3 for animal to human extrapolation with dosimetric adjustment and 10 for human variability) was applied to the $NOAEL_{HED}$.

EPA (IRIS 2009) has derived an oral reference dose (RfD) of 0.003 mg/kg/day based on a NOAEL 0.3 mg/kg/day and LOAEL of 1.5 mg/kg/day for inflammation of the prostate in rats exposed to RDX in the diet for 2 years (U.S. Army 1983a). An uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 to protect against unusually susceptible individuals) was applied to the NOAEL.

## Table 8-1. Regulations and Guidelines Applicable to RDX

| Agency | Description | Information | Reference |
|---|---|---|---|
| INTERNATIONAL | | | |
| Guidelines: | | | |
| IARC | Carcinogenicity classification | No | IARC 2009 |
| WHO | Air quality guidelines | No | WHO 2000 |
| | Drinking water quality guidelines | No | WHO 2006 |
| NATIONAL | | | |
| Regulations and Guidelines: | | | |
| a. Air | | | |
| ACGIH | TLV (8-hour TWA)[a] | 0.5 mg/m$^3$ | ACGIH 2008 |
| | STEL (15-minute TWA) | No | |
| | TLV-basis (critical effect) | Liver damage | |
| AIHA | ERPG values | No | AIHA 2008 |
| EPA | AEGL values | No | EPA 2008a |
| | Hazardous air pollutant | No | EPA 2009b<br>42 USC 7412 |
| NIOSH | REL (10-hour TWA) | 1.5 mg/m$^3$ | NIOSH 2005 |
| | STEL (15-minute) | 3.0 mg/m$^3$ | |
| | IDLH | Not determined | |
| | Target organs | Eyes, skin, and central nervous system | |
| OSHA | PEL (8-hour TWA) for general industry | Vacated[b] | OSHA 1993<br>29 CFR 1910.1000, Final Rule |
| b. Water | | | |
| EPA | Drinking water standards and health advisories | | EPA 2006a |
| | 1-day health advisory for a 10-kg child | 0.1 mg/L | |
| | 10-day health advisory for a 10-kg child | 0.1 mg/L | |
| | DWEL | 0.1 mg/L | |
| | Lifetime | 0.002 mg/L | |
| | $10^{-4}$ Cancer risk | 0.03 mg/L | |
| | National primary drinking water standards | No | EPA 2003 |
| | National recommended water quality criteria | No | EPA 2006b |
| c. Food | | | |
| FDA | EAFUS | No[c] | FDA 2008 |

## Table 8-1. Regulations and Guidelines Applicable to RDX

| Agency | Description | Information | Reference |
|---|---|---|---|
| NATIONAL *(cont.)* | | | |
| d. Other | | | |
| ACGIH | Carcinogenicity classification | A4[d] | ACGIH 2008 |
| EPA | Carcinogenicity classification | Group C[e] | IRIS 2009 |
| | RfC | No | |
| | RfD | $3.0 \times 10^{-3}$ mg/kg/day | |
| | Oral slope factor | 1.1 mg/kg/day$^{-1}$ | |
| | Superfund, emergency planning, and community right-to-know | | |
| | Designated CERCLA hazardous substance | No | EPA 2009c<br>40 CFR 302.4 |
| | Effective date of toxic chemical release reporting | No | EPA 2009d<br>40 CFR 372.65 |
| | TSCA chemical lists and reporting periods | | EPA 2009e<br>40 CFR 712.30 |
| | Effective date | 09/29/2006 | |
| | Reporting date | 11/28/2006 | |
| | TSCA health and safety data reporting | | EPA 2009a<br>40 CFR 716.120 |
| | Effective date | 09/29/2006 | |
| | Sunset date | 11/28/2006 | |
| NTP | Carcinogenicity classification | No | NTP 2005 |

[a]Skin: refers to the potential significant contribution to the overall exposure by the cutaneous route.
[b]On January 19, 1989, OSHA published its final rule on Air Contaminants, which amended 29 CFR 1910.1000 by lowering 212 of OSHA's existing PELs for toxic substances and setting PELs for 164 toxic substances that had been previously unregulated. A PEL value of 1.5 mg/m$^3$ was adopted for RDX in March 1989. However, on July 7, 1992, the Eleventh Circuit Court of Appeals issued a decision in AFL-CIO vs. OSHA that vacated these revised standards.
[c]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[d]A4: not classifiable as a human carcinogen.
[e]Group C: possible human carcinogen, based on hepatocellular adenomas and carcinomas in female B6C3F1 mice.

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; 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; GRAS = Generally Recognized As Safe; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; STEL = short-term expsoure limit; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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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 (Kd)—**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.

**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.

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

**Lethal Dose$_{(LO)}$ ($LD_{Lo}$)—**The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

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

**Lethal Time$_{(50)}$ ($LT_{50}$)—**A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

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

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

**Recommended Exposure Limit (REL)—**A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) 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.

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

**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.

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# 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 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 Environmental Medicine, 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 Environmental Medicine, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-62, Atlanta, Georgia 30333.

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: RDX
CAS Numbers: 121-82-4
Date: August 2011
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Graph Key: 22
Species: Rat

Minimal Risk Level: 0.2 [X] mg/kg/day [ ] ppm

Reference: U.S. Army. 2006. Toxicology study no. 85-XC-5131-03. Subchronic oral toxicity of RDX in rats. Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Experimental design: Groups of six male and six female Sprague-Dawley rats were administered via gavage 0, 2.125, 4.25, 8.5, 17.00, 25.50, 34.00, or 42.5 mg/kg/day as a suspension of RDX/1% methyl-cellulose/0.2% Tween 80 in distilled water 7 days/week for 14 days. Rats were monitored daily for toxic signs and morbidity. Body weights and feed consumption were measured on days 0, 1, 3, 7, and 14. Additional parameters used to assess toxicity included clinical chemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, sodium, potassium, chlorine, cholesterol, creatinine kinase, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides) and hematology (hemoglobin, hematocrit, erythrocytes, mean cell hemoglobin concentration, mean cell volume, mean cell hemoglobin, red blood cell distribution width, total and differential leukocytes, platelets, and mean platelet volume) values, organ weights (brain, heart, liver, kidneys, spleen, adrenals, thymus, epididymides, uterus, testes, ovaries), and gross necropsies.

Effect noted in study and corresponding doses: A significant increase in early deaths was observed at ≥25.5 mg/kg/day. Tremors and convulsions were observed in rats exposed to ≥17 mg/kg/day. In the males exposed to ≥17 mg/kg/day, blood stains around the mouth and nose and low arousal were also observed. Increased arousal, blood around the mouth and nose, barbering, and lacrimation were observed in females exposed to ≥17 mg/kg/day. No signs of neurological alterations were observed in rats exposed to ≤8.5 mg/kg/day. Significant decreases in body weight were observed in male rats exposed to ≥17 mg/kg/day on days 1 and 7, but there were no significant alterations in male body weight at termination. In female rats, significant decreases in body weight gain were observed at ≥34 mg/kg/day on day 1 and in the 8.5 mg/kg/day group on day 14; however, the magnitude of the decreased body weight was less than 10% and no significant alterations were observed at higher dose levels. Significant decreases in food consumption were also observed during the first 7 days of exposure in males and females exposed to ≥8.5 mg/kg/day. Significant decreases in absolute liver weights and liver-to-brain weights and increases in blood cholesterol levels were observed in females exposed to 8.5 mg/kg/day; these effects were not observed at higher dose levels or in males. Due to the lack of dose-response relationships for the alterations in liver weight and blood cholesterol levels, these changes observed in the 8.5 mg/kg/day female group were not considered biologically relevant. No significant alterations in hematological parameters or other clinical chemistry parameters or organ weights were observed.

Dose and end point used for MRL derivation: The MRL is based on a $NOAEL_{HED}$ of 6.45 mg/kg/day for tremors and convulsions in a 14-day study.

[X] NOAEL [ ] LOAEL

PBPK modeling was used to estimate internal dose metrics for brain RDX levels and for estimating HEDs. Code for an RDX oral PBPK model was provided by LM Sweeney along with documentation of parameter values (Sweeney et al. 2012). The Sweeney et al. (2012) model is based on the rat model reported by Krishnan et al. (2009) with modifications made to the gastrointestinal tract parameters, to include two compartments (stomach and small intestine). Sweeney et al. (2012) scaled the rat model to the human and estimated human gastrointestinal absorption and liver metabolism parameter values based on optimization against serum RDX-time profiles from humans accidentally exposed to RDX. Code for the Sweeney et al. (2012) model was implemented in acsLX v 3.0.1.6, without modification to the human or rat parameter values. Performance of the implementation was verified by comparing output to plots shown in Figure 2-4 of Sweeney et al. (2012).

The model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to simulate external rat dosages used in relevant bioassays and to predict the corresponding internal dose metric, peak concentration of RDX in brain ($CB_{peak}$) and mean concentration of RDX in brain ($CB_{mean}$).

2. Gavage doses (mg/kg/day) were assumed to be delivered as a single bolus each day, at the exposure frequency (days/week) used in the bioassay.

3. Rat model simulations were carried out for 14 days for acute exposures.

4. Rat body weights used in the simulations were the time-weighted average (TWA) body weights for each dose group.

5. The human model was used to predict the daily dosage (mg/kg/day) corresponding to the NOAEL for peak brain concentration in the rat.

6. A body weight of 70 kg was assumed for humans.

7. Daily doses (mg/kg/day) in humans were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, 7 days/week.

8. Human model simulations were carried out for 14 days for acute exposures.

The peak and mean brain concentrations for each dose are presented in Table A-1.

## Table A-1. Estimated Peak and Mean Brain Concentrations in Rats Administered RDX Via Gavage 7 Days/Week for 14 Days

| Dose (mg/kg/day) | TWA body weight (kg) | Peak brain concentration (mg/L) | Mean brain concentration (mg/L) |
|---|---|---|---|
| Males | | | |
| 0 | 0.2039 | 0 | 0 |
| 2.13 | 0.2044 | 1.602 | 0.6645 |
| 4.25 | 0.2017 | 3.198 | 1.3232 |
| 8.5 | 0.1915 | 6.351 | 2.6018 |
| 17 | 0.1826 | 12.619 | 5.1232 |
| 25.5 | 0.1886 | 19.012 | 7.7666 |
| 34 | 0.1693[a] | 24.979 | 9.9956 |
| 42.5 | 0.1683[a] | 31.198 | 12.4702 |
| Females | | | |
| 0 | 0.1403 | 0 | 0 |
| 2.13 | 0.1375 | 1.518 | 0.5835 |
| 4.25 | 0.1397 | 3.042 | 1.1732 |
| 8.5 | 0.131 | 6.033 | 2.2974 |
| 17 | 0.1347 | 12.1211 | 4.6369 |
| 25.5 | 0.1374 | 18.214 | 7.0007 |
| 34 | 0.1250[a] | 23.982 | 9.0492 |
| 42.5 | 0.1262[a] | 30.016 | 11.3470 |

[a]Day 1 body weight used due to high mortality (100% mortality on day 1 in 42.5 mg/kg/day males and females and 34 mg/kg/day females and 83% mortality on day 1 in 34 mg/kg/day males)

TWA = time-weighted average

Source: U.S. Army 2006

The acute-duration oral MRL was derived using the NOAEL/LOAEL approach; the lack of incidence data for the neurological effects precluded using a benchmark dose approach. The U.S. Army (2006) study identified a NOAEL of 8.5 mg/kg/day and LOAEL of 17 mg/kg/day for neurological effects. The PBPK model was used to predict peak brain RDX concentrations and mean brain RDX concentrations associated with these dose levels. In animals dosed with 8.5 mg/kg/day, the model predicted peak brain concentrations of 6.351 mg/L in males and 6.033 mg/L in females and mean brain concentrations of 2.602 and 2.297 mg/L in males and females, respectively. Mechanistic data provide strong support that the mode of action for seizures involves binding to GABA receptors and there is a direct relationship between RDX levels in the brain and the onset of seizures (Gust et al. 2009; Williams et al. 2011). However, there are insufficient data to determine whether peak brain RDX concentration or mean brain RDX concentration is the most appropriate internal dose metric. As presented in Table A-2, a comparison of the NOAEL and LOAEL values for seizures in rats exposed for intermediate or chronic durations suggests that peak brain concentration may be the most appropriate internal dose metric. The mean brain concentrations are similar for rats exposed to 8 mg/kg/day for 90 days or 2 years; however, seizures/convulsions were observed at this dose level in the 90-day study, but not in the 2-year study. In contrast to the mean concentrations, the peak brain concentration in the 90-day study was 34% higher

than in the 2-year study; this difference in peak concentrations may explain the apparent difference in seizure threshold. Thus, peak brain concentration was selected as the internal dose metric for derivation of the acute-duration oral MRL. Since the U.S. Army (2006) study did not identify gender-specific differences in RDX sensitivity, the peak brain concentrations were averaged for the male and females rats. In the rats administered 8.5 mg/kg/day RDX, the average peak brain RDX concentration was predicted to be 6.192 mg/L. This peak brain concentration was used to predict a HED of 6.455 mg/kg/day using the PBPK model.

## Table A-2. Comparison of NOAEL and LOAEL Values Using Different Dose Metrics Following Intermediate- and Chronic-Duration Exposure to RDX

| | Intermediate exposure[a] (U.S. Army 2006) | Chronic exposure[b] (U.S. Army 1983a) |
|---|---|---|
| NOAEL[c] | | |
| Administered dose | 4 mg/kg/day | 8 mg/kg/day |
| Peak brain concentration | 2.923 mg/L | 4.051 mg/L |
| Mean brain concentration | 1.308 mg/L | 2.959 mg/L |
| LOAEL[c] | | |
| Administered dose | 8 mg/kg/day | 40 mg/kg/day |
| Peak brain concentration | 6.013 mg/L | 18.694 mg/L |
| Mean brain concentration | 2.615 mg/L | 14.403 mg/L |

[a]RDX administered via gavage, 7 days/week for 90 days
[b]RDX administered via the diet for 2 years
[c]Average of male and female values

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans with dosimetric adjustments
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

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? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: Human case reports have noted convulsions and seizures in individuals ingesting RDX (Hollander and Colbach 1969; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986). Several acute toxicity studies have reported convulsions, seizures, or tremors in rats at doses slightly higher than the LOAEL of 17 mg/kg/day identified in the U.S. Army (2006) study. These LOAEL values are 20 mg/kg/day in two gestational exposure studies (U.S. Army 1980b, 1986d) and 25 mg/kg in rats administered a single gavage dose (Burdette et al. 1988). In addition, decreases in motor activity and learning were observed in rats receiving a single gavage dose of 12.5 mg/kg/day (U.S. Army 1985b).

Although the potential for systemic effects has not been well investigated following acute exposure, intermediate-duration studies (U.S. Army 1980b, 1983a, 2006; U.S. Navy 1974b) provide support that neurotoxicity is the most sensitive effect of RDX.

Agency Contacts (Chemical Managers): Henry Abadin

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: RDX
CAS Numbers: 121-82-4
Date: August 2011
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Graph Key: 50
Species: Rat

Minimal Risk Level: 0.1 [X] mg/kg/day [ ] ppm

Reference: U.S. Army. 2006. Toxicology study no. 85-XC-5131-03. Subchronic oral toxicity of RDX in rats. Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Experimental design: Groups of 10 male and 10 female F344 rats were administered via gavage 0, 4, 8, 10, 12, or 15 mg/kg/day as a suspension of RDX/1% methylcellulose/0.2% Tween 80 in distilled water 7 days/week for 90 days. Rats were monitored weekly for toxic signs and FOB observations (home-cage, hand held, and open arena observations); body weights and feed consumption were also measured weekly. Additional parameters used to assess toxicity included neurobehavioral tests after week 11 (motor activity, grip strength, and sensory reactivity to different types of stimuli), ophthalmic examination, urinalysis (volume, color, appearance, pH, specific gravity, glucose, bilirubin, urobilinogen, ketone, blood, protein, nitrite, leukocytes), clinical chemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, sodium, potassium, chlorine, cholesterol, creatinine kinase, creatinine, glucose, lactate dehydrogenase, total bilirubin, total protein, triglycerides), hematology (hemoglobin, hematocrit, erythrocytes, mean cell hemoglobin concentration, mean cell volume, mean cell hemoglobin, red blood cell distribution width, total and differential leukocytes, platelets, and mean platelet volume) values, coagulation (average and activated prothrombin time), organ weights (brain, heart, liver, kidneys, spleen, adrenals, thymus, epididymides, uterus, testes, ovaries), gross necropsies, and histopathological examination of major tissues and organs from rats exposed to 0 or 15 mg/kg/day. In addition, potential immunotoxicity was assessed using the following tests: red and white blood cell populations and spleen and thymus relative organ weights, cellularity as a proportion of organ weight, and proportion of cell surface markers.

Effect noted in study and corresponding doses: Increased mortality was observed at ≥8 mg/kg/day; the number of preterm deaths were 2/20, 5/20, 8/20, and 7/20 in the 8, 10, 12, and 15 mg/kg/day groups, respectively. Convulsions were observed in most animals dying early. Transient clinical signs included changes in arousal, blepharosis, increased salivation, blood stains around mouth and nose, rough haircoat, tremors, and convulsions; the incidence and severity of these effects increased with dose. Neuromuscular effects were observed within the first week of exposure in the higher dose groups and persisted throughout the study. Increased arousal was observed in 25, 40, and 100% of rats in the 10, 12, and 15 mg/kg/day groups; convulsions were observed in 15, 30, 65, and 60% of rats in the 8, 10, 12, and 15 mg/kg/day groups, respectively; and tremors were observed in 10 and 20% of rats in the 12 and 15 mg/kg/day groups. Increased urine volume was observed in females exposed to 12 or 15 mg/kg/day; the investigators noted that the increased urine volume may be related to the palatability of the suspension since higher dose animals were frequently observed drinking immediately after dosing. Significant decreases in body weight gain were observed in the male rats; however, body weights were typically within 10% of controls. In the females, significant increases in body weight were observed; at termination, the females in the 10, 12, and 15 mg/kg/day groups weighed at least 14% more than controls.

Significant alterations in organ weights were observed in male rats; these included increased brain weight at 12 and 15 mg/kg/day, decreased relative (to body weight and brain weight) testes weight at ≥10 mg/kg/day, and decreased relative (to brain weight) epididymis weight at ≥8 mg/kg/day. In the females, significant alterations in organ weights included increased spleen, liver, and kidney weights at 10, 12 (spleen only), or 15 mg/kg/day; relative brain weight at ≥10 mg/kg/day; and increased relative (to brain) kidney, liver, and spleen weights at 10 and 15 mg/kg/day. Significant increases in mean cell volume were observed at 8 (males only), 10, and 12 mg/kg/day and significant decreases in cholesterol levels were observed in males exposed to ≥8 mg/kg/day. No significant increases in the incidence of histopathological alterations were observed. A significant increase in abnormal skin appearance (stained haircoat) was observed in females exposed to 15 mg/kg/day during week 12. The presence of barbering was significantly increased in females exposed to 15 mg/kg/day during weeks 9 and 12. No RDX related alterations in immunological parameters were observed. Although the incidence of convulsions was not statistically significant at 8 mg/kg/day, this dose level, which likely falls just below the NOAEL/LOAEL boundary, was considered a LOAEL due the seriousness of the effect.

Dose and end point used for MRL derivation: The $BMDL_{HED}$ of 4.1308 mg/kg/day for convulsions was used as the point of departure for the MRL.

[ ] NOAEL [ ] LOAEL [X] $BMDL_{10}$

PBPK modeling was used to estimate internal dose metrics for brain RDX levels and for estimating HEDs. Code for an RDX oral PBPK model was provided by LM Sweeney along with documentation of parameter values (Sweeney et al. 2012). The Sweeney et al. (2012) model is based on the rat model reported by Krishnan et al. (2009) with modifications made to the gastrointestinal tract parameters, to include two compartments (stomach and small intestine). Sweeney et al. (2012) scaled the rat model to the human and estimated human gastrointestinal absorption and liver metabolism parameter values based on optimization against serum RDX-time profiles from humans accidentally exposed to RDX. Code for the Sweeney et al. (2012) model was implemented in acsLX v 3.0.1.6, without modification to the human or rat parameter values. Performance of the implementation was verified by comparing output to plots shown in Figure 2-4 of Sweeney et al. (2012).

The model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to simulate external rat dosages used in relevant bioassays and to predict the corresponding internal dose metrics, peak concentration of RDX in brain ($CB_{peak}$) and mean concentration of RDX in brain ($CB_{mean}$).

2. Gavage doses (mg/kg/day) were assumed to be delivered as a single bolus each day, at the exposure frequency (days/week) used in the bioassay.

3. Rat model simulations were carried out until steady state had been achieved for intermediate-duration exposures.

4. Rat body weights used in the simulations were the TWA body weights for each dose group.

5. The human model was used to predict the daily dosage (mg/kg/day) corresponding to the BMDL for peak brain concentration in the rat.

6. A body weight of 70 kg was assumed for humans.

7. Daily doses (mg/kg/day) in humans were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, 7 days/week.

8. Human model simulations were carried out until steady state had been achieved for intermediate-duration exposures.

The peak and mean brain concentrations for each dose are presented in Table A-3.

**Table A-3. Estimated Peak and Mean Brain Concentrations in Rats Administered RDX Via Gavage 7 Days/Week for 90 Days**

| Dose (mg/kg/day) | TWA body weight (kg) | Peak brain concentration (mg/L) | Mean brain concentration (mg/L) |
|---|---|---|---|
| Males | | | |
| 0 | 0.2558 | 0 | 0 |
| 4 | 0.2435 | 3.090 | 1.3947 |
| 8 | 0.2362 | 6.154 | 2.7616 |
| 10 | 0.2418 | 7.718 | 3.4787 |
| 12 | 0.2446 | 9.277 | 4.1903 |
| 15 | 0.2579 | 11.683 | 5.3301 |
| Females | | | |
| 0 | 0.1642 | 0 | 0 |
| 4 | 0.1626 | 2.923 | 1.2209 |
| 8 | 0.1682 | 5.873 | 2.4692 |
| 10 | 0.1714 | 7.359 | 3.1057 |
| 12 | 0.1722 | 8.837 | 3.7325 |
| 15 | 0.1849 | 11.154 | 4.7764 |

TWA = time-weighted average

Source: U.S. Army 2006

The intermediate-duration oral MRL was derived using a benchmark dose modeling approach. Peak brain concentration and mean brain concentration were considered potential internal dose metrics for the benchmark dose modeling. Mechanistic data provide strong support that the mode of action for seizures involves binding to GABA receptors and there is a direct relationship between RDX levels in the brain and the onset of seizures (Gust et al. 2009; Williams et al. 2011). However, there are insufficient data to determine whether peak brain RDX concentration or mean brain RDX concentration is the most appropriate internal dose metric. As presented in Table A-4, the empirical data for seizures/convulsions appears to support using peak brain concentration as the internal dose metric. The mean brain concentrations are similar for rats exposed to 8 mg/kg/day for 90 days or 2 years; however, seizures/convulsions were observed at this dose level in the 90-day study, but not in the 2-year study. In contrast to the mean concentrations, the peak brain concentration in the 90-day study was 34% higher than in the 2-year study; this difference in peak concentrations may explain the apparent difference in seizure threshold.

## Table A-4. Comparison of NOAEL and LOAEL Values Using Different Dose Metrics Following Intermediate- and Chronic-Duration Exposure to RDX

| | Intermediate exposure[a] (U.S. Army 2006) | Chronic exposure[b] (U.S. Army 1983a) |
|---|---|---|
| NOAEL[c] | | |
| Administered dose | 4 mg/kg/day | 8 mg/kg/day |
| Peak brain concentration | 2.923 mg/L | 4.051 mg/L |
| Mean brain concentration | 1.308 mg/L | 2.959 mg/L |
| LOAEL[c] | | |
| Administered dose | 8 mg/kg/day | 40 mg/kg/day |
| Peak brain concentration | 6.013 mg/L | 18.694 mg/L |
| Mean brain concentration | 2.615 mg/L | 14.403 mg/L |

[a]RDX administered via gavage, 7 days/week for 90 days.
[b]RDX administered via the diet for 2 years.
[c]Average of male and female values.

Data for the incidence of convulsions (summarized in Table A-5) were fit to all available dichotomous models in the EPA Benchmark Dose Software (BMDS) (version 2.1.2) using the extra risk option and using peak brain RDX concentration as the dose metric. Since the study did not identify gender-specific differences in RDX sensitivity, the peak brain concentrations were averaged for the male and female rats and these combined values were used for benchmark dose modeling. Adequate model fit was judged by three criteria: $\chi^2$ goodness-of-fit p-value ($p>0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. BMDs and lower bounds on the BMD (BMDL) associated with a BMR of 10% extra risk were calculated for all models and are presented in Table A-6. As assessed by the $\chi^2$ goodness-of-fit statistic, all of the models with the exception of the quantal linear and 1-degree polynomial models provided adequate fit to the data. Among all of the models providing adequate fit to the data, the BMDL from the model with the lowest Akaike's Information Criteria (AIC) was chosen. The log-probit model provided the best fit to the convulsion incidence data and is presented in Figure A-1. The BMDL of 3.9627 mg/L was used to predict a HED of 4.131 mg/kg/day using the PBPK model.

## Table A-5. Incidence of Convulsions in Male and Female Fischer 344 Rats Administered RDX 7 Days/Week for 90 Days

| Dose (mg/kg/day) | Peak brain concentration (mg/L) | Incidence |
|---|---|---|
| 0 | 0 | 0/20 |
| 4 | 3.005 | 0/20 |
| 8 | 6.013 | 3/20 |
| 10 | 7.539 | 6/20 |
| 12 | 9.057 | 13/20 |
| 15 | 11.419 | 12/20 |

Source: U.S. Army 2006

## Table A-6. Model Predictions for the Incidence of Convulsions in Rats Administered RDX via Gavage for 90 Days Using Peak Brain Concentration as the Internal Dose Metric

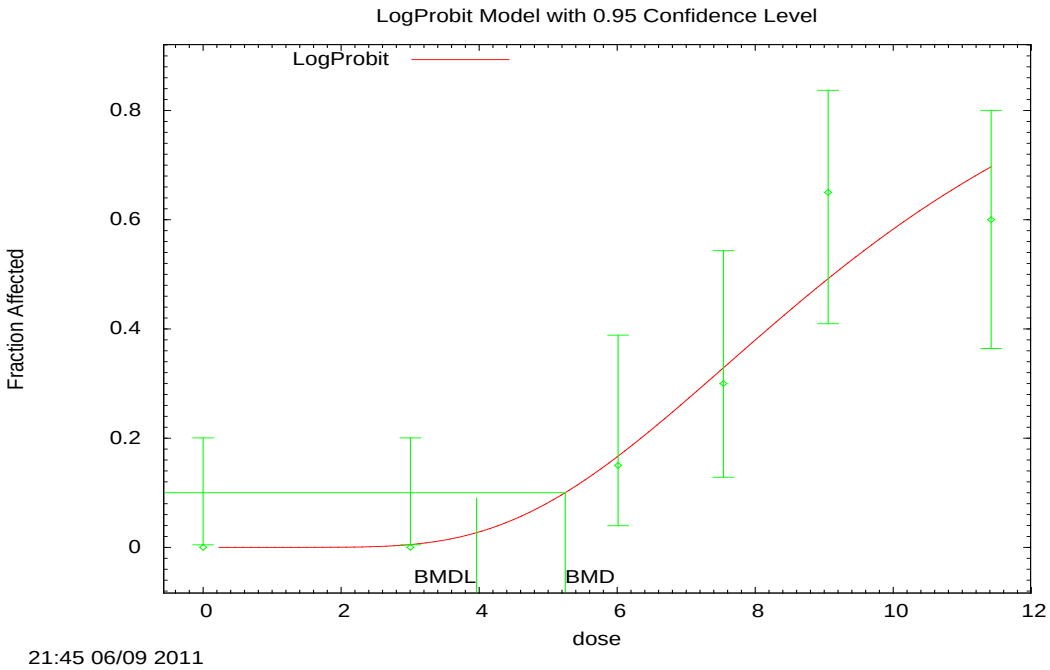
| Model | $\chi^2$ Goodness of fit p-value[a] | AIC | $BMD_{10}$ (mg/L) | $BMDL_{10}$ (mg/L) |
|---|---|---|---|---|
| Gamma[b] | 0.4648 | 101.924 | 5.17803 | 3.79207 |
| Logistic | 0.2121 | 104.808 | 5.14052 | 3.99687 |
| LogLogistic | 0.4945 | 101.781 | 5.18956 | 3.8386 |
| **LogProbit** | **0.5406** | **101.353** | **5.24819** | **3.9627** |
| Multistage (1-degree polynomial)[c] | 0.3663 | 111.445 | 3.75703 | 2.82257 |
| Multistage (2-degree polynomial[c] | 0.0383 | 102.99 | NA | NA |
| Probit | 0.2696 | 103.851 | 5.11305 | 3.88122 |
| Weibull[b] | 0.352 | 103.06 | 4.87416 | 3.43247 |
| Quantal-Linear | 0.0383 | 111.445 | NA | NA |

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Betas restricted to ≥0.

AIC = Akaike's Information Criterion; BMD = maximum likelihood estimate of the dose associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD; NA = not applicable, model does not provide adequate fit to the data

Source: U.S. Army 2006

**Figure A-1. Fit of Log Probit Model to Data on the Incidence of Convulsions in Rats Administered RDX via Gavage for 90 Days Using Peak Brain RDX Concentration as the Dose Metric**




Source: U.S. Army 2006

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans with dosimetric adjustments
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

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? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: No human studies have examined the toxicity of RDX following intermediate-duration exposure. Several animal studies have reported neurological effects, primarily convulsions, seizures, and/or tremors in rats at doses of ≥8 mg/kg/day (U.S. Army 1983a, 2006; von Oettingen et al. 1949), monkeys at 10 mg/kg/day (U.S. Navy 1974b), and dogs at 50 mg/kg/day (von Oettingen et al. 1949). Hyperactivity was noted in rats exposed to 100 mg/kg/day (Levine et al. 1981, 1990). The results of the U.S. Army (2006) study suggest that there is a steep dose-response curve for seizure induction. The occurrences of seizures were 0% at 4 mg/kg/day, 20–30% at 8 mg/kg/day, 45–50% at 10 mg/kg/day, and 80–90% at 12 or 15 mg/kg/day.

In addition to these neurological effects, less serious adverse health effects have been observed at similar or higher dose levels. Several studies have found changes in serum chemistry parameters suggestive of

impaired liver function, although histological alterations were not generally found in the liver. Decreases in serum cholesterol and/or triglycerides were observed at ≥8 mg/kg/day (U.S. Army 1983a, 2006; Levine et al. 1981) and decreases in serum alanine aminotransferase levels were observed at 28 mg/kg/day (U.S. Army 1980b). The magnitude of these alterations was small and not likely to be biologically significant. Minor hematological effects (small decreases in erythrocyte and hemoglobin levels) were observed in rats exposed to 40 mg/kg/day (U.S. Army 1983a) and mice exposed to 160 mg/kg/day (U.S. Army 1980b); however, other studies have not found significant alterations in hematological parameters (U.S. Army 1980b, 2006; von Oettingen et al. 1949). Emesis was observed in monkeys administered via gavage 10 mg/kg/day for 90 days (U.S. Navy 1974b); the incidence in monkeys administered 1 mg/kg/day was not considered to be different from the controls. There is limited evidence that RDX is a reproductive toxicant. Spermatic granuloma in the prostrate was observed in rats exposed to 40 mg/kg/day for 6 months (U.S. Army 1983a). Decreases in $F_2$ pup body weight and increases in the incidence of renal cysts were observed at 16 mg/kg/day and an increase in the number of stillbirths and decreased pup survival were observed in the $F_1$ generation at 50 mg/kg/day was observed in a two-generation study in rats (U.S. Army 1980b).

Agency Contacts (Chemical Managers): Henry Abadin

APPENDIX A

# MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: RDX
CAS Numbers: 121-82-4
Date: August 2011
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key: 65
Species: Rat

Minimal Risk Level: 0.1 [X] mg/kg/day [ ] ppm

Reference: U.S. Army. 1983a. Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat: Phase V. Vol. 1. Frederick, MD: U.S. Army Medical Research and Development Command. ADA160774. (author: Levine BS et al.)

Experimental design: Groups of male and female Fischer 344 rats (75/sex/group) were exposed to 0, 0.3, 1.5, 8.0, or 40.0 mg/kg/day RDX in the diet for 2 years. Ten animals/sex/dose were sacrificed during weeks 27 and 53. The following parameters were used to assess toxicity: daily observations; ophthalmic examinations during weeks 2, 25, 51, 76, and 103; hematology (hematocrit, hemoglogin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, erythrocyte count, total and differential leukocyte count, and platelet count) and clinical chemistry (glucose, blood urea nitrogen, alanine aminotransferase, bilirubin, creatinine phosphokinase, lactic dehydrogenase, alkaline phosphatase, triglycerides, total cholesterol, total protein, albumin, globulin, sodium, potassium, chloride, and calcium levels) of blood samples collected during weeks 13, 26, 52, 78, and 104; organ weights (adrenal, brain, heart, kidneys, liver, ovaries, spleen, and testes), and complete histopathology of major tissues and organs of rats in the 0 or 40.0 mg/kg/day groups, and histopathological examination of the brain, gonads, heart, liver, kidneys, spleen, and spinal cord of rats in the 0.3, 1.5, and 8.0 mg/kg/day groups. Actual RDX doses were within 3% of the intended dose.

Effect noted in study and corresponding doses: Deaths were observed at 40 mg/kg/day; 88% of males and 41% of females died by week 88. The mean survival time for the 40 mg/kg/day males was 14.6 months compared with 22.3 months for the control males. A 20.6 month survival time was seen for the 40 mg/kg/day females vs. 22.0 months for the control females at 40 mg/kg/day. A significant decrease in survival time was also observed in the males exposed to 1.5 mg/kg/day (21.0 months); however, no alterations in survival time was observed in the females exposed to 1.5 mg/kg/day (22.2 months) or in the males (22.2 months) or females (22.4 months) exposed to 8 mg/kg/day. Additionally, there were no significant differences in mortality incidence in the 1.5 or 8 mg/kg/day groups, as compared to controls. Decreased body weight gain was observed in males (20–30%) and females (10–15%) exposed to 40.0 mg/kg/day; significant decreases in body weight gain were also observed at 8.0 mg/kg/day, but the body weight was within 5% of controls. Slight, but significant, reductions in food intake were observed in males at 40.0 mg/kg/day. Tremors and convulsions were observed prior to death at 40 mg/kg/day beginning after 26 weeks of exposure. Animals were hyperreactive to approach and had increased fighting; hyperreactivity was first observed after 9 weeks of exposure to 40 mg/kg/day. No adverse clinical signs were noted for the lower dose groups. Significant decreases in hemoglobin and erythrocyte counts were observed in the 40 mg/kg/day group beginning at week 26; the study investigators noted that the anemic state was considered slight and there was no evidence of physiologic compensatory responses. Thrombocytosis was observed in rats exposed to 40 mg/kg/day and elevated platelet counts were

observed in 8 mg/kg/day males during weeks 13 and 26. Significant decreases in blood glucose, total cholesterol, and triglyceride levels were observed in the 40 mg/kg/day group starting at week 13. Significant decreases in serum alanine aminotransferase levels were observed in males exposed to 8 or 40 mg/kg/day at weeks 26 and 52 and in females at 40 mg/kg/day at week 26. Other clinical chemistry alterations included decreases in globulin and albumin levels at weeks 52 and 78 and increases in serum potassium levels at weeks 26, 52, and 78. A significant increase in the incidence of cataracts was observed in females in the 40 mg/kg/day group during weeks 78 and 104. Splenic extramedullary hematopoiesis and spermatic granuloma of the prostate were observed in rats exposed to 40 mg/kg/day for 6 months. At 1 year, histological alterations in the urinary bladder (luminal distention and cystitis), kidneys (medullary papillary necrosis), and testes (germinal cell degeneration, enlarged seminal vesicles) were observed in males exposed to 40 mg/kg/day and in the spleen (enlarged dark-red spleens with histological evidence of sinusoidal congestion) of males and females exposed to 40 mg/kg/day. The following effects were observed at 2 years: suppurative inflammation of the prostate in the 1.5, 8, and 40 mg/kg/day groups; renal medullary papillar necrosis, renal pyelitis, and urinary bladder luminal distension and cystitis in males exposed to 40 mg/kg/day; splenic extramedullary hematopoiesis in female rats exposed to 40 mg/kg/day; and hemosiderin-like pigment in males exposed to 1.5, 8, or 40 mg/kg/day. In the absence of altered hematological parameters or other effects on the spleen, the increased pigment levels observed at 1.5 or 8 mg/kg/day were not considered adverse.

Dose and end point used for MRL derivation: The MRL is based on a $NOAEL_{HED}$ of 4.223 mg/kg/day for tremors and convulsions in a 2-year study.

[X] NOAEL [ ] LOAEL

PBPK modeling was used to estimate internal dose metrics for brain RDX levels and for estimating HEDs. Code for an RDX oral PBPK model was provided by LM Sweeney along with documentation of parameter values (Sweeney et al. 2012). The Sweeney et al. (2012) model is based on the rat model reported by Krishnan et al. (2009) with modifications made to the gastrointestinal tract parameters, to include two compartments (stomach and small intestine). Sweeney et al. (2012) scaled the rat model to the human and estimated human gastrointestinal absorption and liver metabolism parameter values based on optimization against serum RDX-time profiles from humans accidentally exposed to RDX. Code for the Sweeney et al. (2012) model was implemented in acsLX v 3.0.1.6, without modification to the human or rat parameter values. Performance of the implementation was verified by comparing output to plots shown in Figure 2-4 of Sweeney et al. (2012).

The model was used for interspecies extrapolation of rat internal dosimetry to humans using the following procedure:

1. The rat model was used to simulate external rat dosages used in relevant bioassays and to predict the corresponding internal dose metric, peak concentration of RDX in brain ($CB_{peak}$) and mean concentration of RDX in brain ($CB_{mean}$).

2. Dietary doses (mg/kg/day) were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, at the exposure frequency (days/week) used in the bioassay.

3. Rat model simulations were carried out until steady state had been achieved for chronic-duration exposures.

4. Rat body weights used in the simulations were the TWA body weights for each dose group.

5. The human model was used to predict the daily dosage (mg/kg/day) corresponding to the NOAEL for peak brain concentration in the rat.

6. A body weight of 70 kg was assumed for humans.

7. Daily doses (mg/kg/day) in humans were assumed to be delivered in 12 consecutive hourly doses, separated by 12-hour intervals, 7 days/week.

8. Human model simulations were carried out until steady state had been achieved for chronic-duration exposures.

The peak brain concentrations for each dose are presented in Table A-7.

## Table A-7. Estimated Peak and Mean Brain Concentrations in Rats Administered RDX Via the Diet for 2 Years

| Dose (mg/kg/day) | TWA body weight (kg) | Peak brain concentration (mg/L) | Mean brain concentration (mg/L) |
|---|---|---|---|
| Males | | | |
| 0 | 0.3889 | 0 | 0 |
| 0.3 | 0.3904 | 0.165 | 0.122 |
| 1.5 | 0.3848 | 0.823 | 0.607 |
| 8 | 0.373 | 4.344 | 3.205 |
| 40 | 0.3244 | 20.885 | 15.304 |
| Females | | | |
| 0 | 0.2302 | 0 | 0 |
| 0.3 | 0.2297 | 0.142 | 0.102 |
| 1.5 | 0.2278 | 0.707 | 0.511 |
| 8 | 0.2248 | 3.763 | 2.712 |
| 40 | 0.2218 | 18.694 | 13.502 |

TWA = time-weighted average

Source: U.S. Army 1983a

The U.S. Army (1983a) study identified a NOAEL of 8 mg/kg/day and LOAEL of 40 mg/kg/day for tremors and convulsions in rats exposed to RDX in the diet for 2 years. A chronic-duration oral MRL was derived using the NOAEL/LOAEL approach; benchmark dose modeling could not be utilized because the investigators did not report incidence data for neurological signs. The NOAEL from the U.S. Army (1983a) study corresponds to peak brain concentrations of 4.344 and 3.763 mg/L in males and females, respectively, and mean brain RDX concentrations of 3.205 and 2.712 mg/L in males and females, respectively. Mechanistic data provide strong support that the mode of action for seizures involves binding to GABA receptors and there is a direct relationship between RDX levels in the brain and the onset of seizures (Gust et al. 2009; Williams et al. 2011). However, there are insufficient data to determine whether peak brain RDX concentration or mean brain RDX concentration is the most appropriate internal dose metric. As presented in Table A-8, a comparison of the NOAEL and LOAEL values for seizures in rats exposed for intermediate or chronic durations suggests that peak brain

concentration may be the most appropriate internal dose metric. The mean brain concentrations are similar for rats exposed to 8 mg/kg/day for 90 days or 2 years; however, seizures/convulsions were observed at this dose level in the 90-day study, but not in the 2-year study. In contrast to the mean concentrations, the peak brain concentration in the 90-day study was 34% higher than in the 2-year study; this difference in peak concentrations may explain the apparent difference in seizure threshold. Thus, peak brain concentration was selected as the internal dose metric for derivation of the acute-duration oral MRL. Since the U.S. Army (1983a) study did not identify gender-specific differences in RDX sensitivity, the peak brain concentrations were averaged for the male and females rats. The average peak brain concentration of 4.051 mg/L was used to predict a HED of 4.223 mg/kg/day using the PBPK model.

## Table A-8. Comparison of NOAEL and LOAEL Values Using Different Dose Metrics Following Intermediate- and Chronic-Duration Exposure to RDX

| | Intermediate exposure[a] (U.S. Army 2006) | Chronic exposure[b] (U.S. Army 1983a) |
|---|---|---|
| NOAEL[c] | | |
| Administered dose | 4 mg/kg/day | 8 mg/kg/day |
| Peak brain concentration | 2.923 mg/L | 4.051 mg/L |
| Mean brain concentration | 1.308 mg/L | 2.959 mg/L |
| LOAEL[c] | | |
| Administered dose | 8 mg/kg/day | 40 mg/kg/day |
| Peak brain concentration | 6.013 mg/L | 18.694 mg/L |
| Mean brain concentration | 2.615 mg/L | 14.403 mg/L |

[a]RDX administered via gavage, 7 days/week for 90 days.
[b]RDX administered via the diet for 2 years.
[c]Average of male and female values.

Uncertainty Factors used in MRL derivation:

[ ] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans with dosimetric adjustments
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? Not applicable.

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? Not applicable.

Other additional studies or pertinent information that lend support to this MRL: No human studies have examined the chronic toxicity of RDX following oral exposure. A number of human case reports have noted convulsions and seizures in individuals ingesting RDX (Hollander and Colbach 1969; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986). The chronic oral toxicity of RDX has been evaluated in two rat studies (U.S. Army 1983a; U.S. Navy 1976) and a mouse study (U.S. Army 1984c). A number of adverse health effects have been observed in rats exposed to 40 mg/kg/day including tremors, convulsions, and hyperresponsiveness; decreased hematocrit,

hemoglobin, and erythrocyte levels; hepatomegaly and decreased serum cholesterol and triglycerides; renal papillary necrosis and increased blood urea nitrogen levels; testicular degeneration; and cataracts (females only) (U.S. Army 1983a). This dose was also associated with an 88% mortality rate. In addition to these effects, significant increases in the incidence of suppurative inflammation were observed in the prostate of rats exposed to ≥1.5 mg/kg/day (U.S. Army 1983a). U.S. Army (2006) noted that inflammation of the prostate gland is a common condition in older rodents and is generally not due to toxicity; additionally, the prostate effects in the U.S. Army (1983a) study were predominantly found in rats dying early.

In the second rat study, no adverse effects were observed at doses as high as 10 mg/kg/day (U.S. Navy 1976). This study did not include a histological examination of the prostate, and the animals were monitored weekly for overt signs of toxicity. In mice, increases in serum cholesterol levels were observed in females exposed to 35 mg/kg/day and increased relative kidney weights and cytoplasmic vacuolization in the kidney were observed at 100 mg/kg/day. The NOAEL for the hepatic effects was 7 mg/kg/day.

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# APPENDIX B. USER'S GUIDE

## Chapter 1

### Public Health Statement

This chapter of the profile is a health effects summary written in nontechnical 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.

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.

**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 (<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").

(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 $mg/m^3$ 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.

(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**

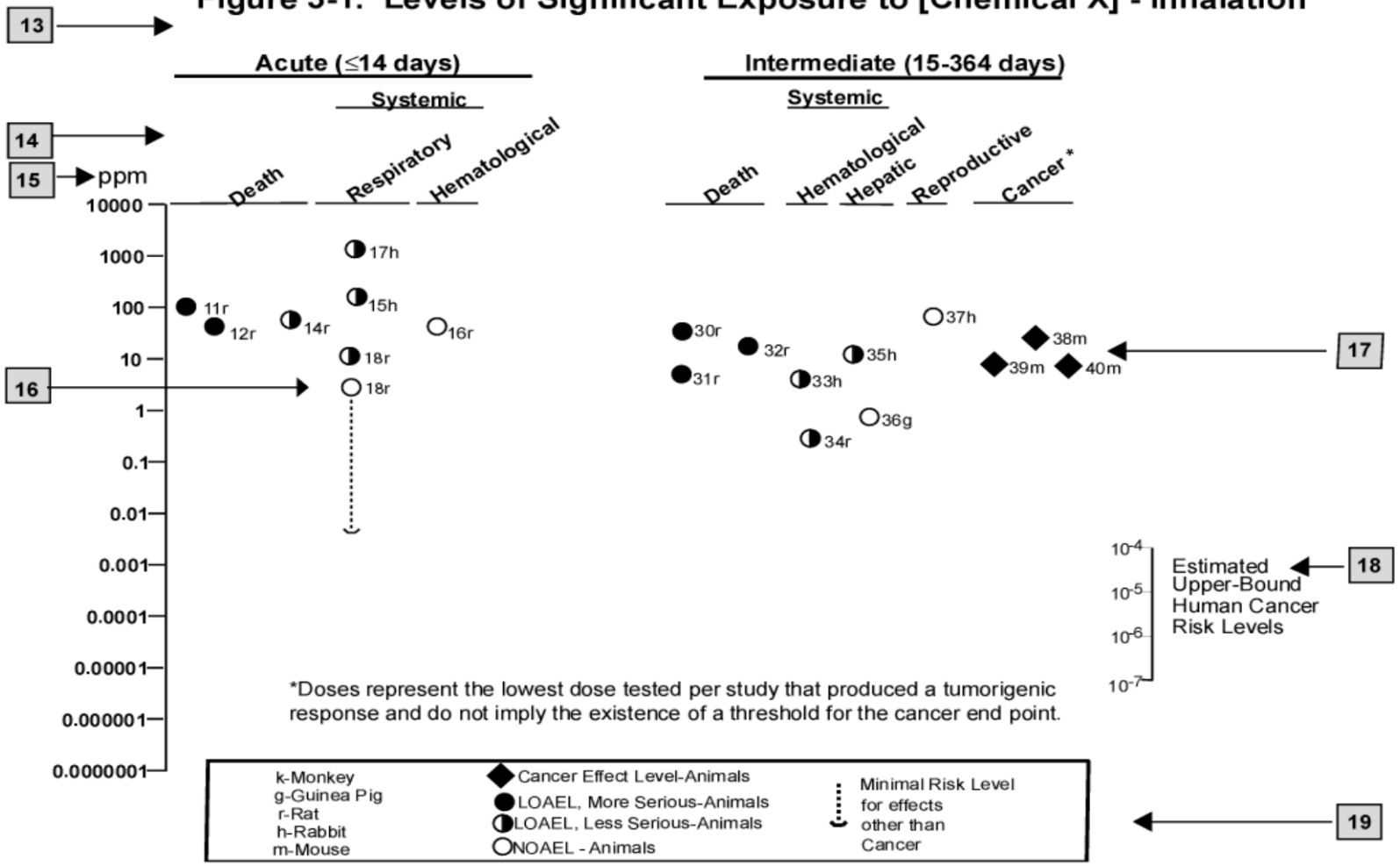
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

| Key to figure[a] | Species | Exposure frequency/ duration | System | NOAEL (ppm) | LOAEL (effect) Less serious (ppm) | LOAEL (effect) Serious (ppm) | Reference |
|---|---|---|---|---|---|---|---|
| 2 → INTERMEDIATE EXPOSURE | | | | | | | |
| | 5 | 6 | 7 | 8 | 9 | | 10 |
| 3 → Systemic | ↓ | ↓ | ↓ | ↓ | ↓ | | ↓ |
| 18 | Rat | 13 wk<br>5 d/wk<br>6 hr/d | Resp | 3[b] | 10 (hyperplasia) | | Nitschke et al. 1981 |
| 4 → CHRONIC EXPOSURE | | | | | | | |
| Cancer | | | | | 11 | | |
| | | | | | ↓ | | |
| 38 | Rat | 18 mo<br>5 d/wk<br>7 hr/d | | | 20 | (CEL, multiple organs) | Wong et al. 1982 |
| 39 | Rat | 89–104 wk<br>5 d/wk<br>6 hr/d | | | 10 | (CEL, lung tumors, nasal tumors) | NTP 1982 |
| 40 | Mouse | 79–103 wk<br>5 d/wk<br>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 |
| DOT | Department of Transportation |
| DOT/UN/ NA/IMDG | Department of Transportation/United Nations/ 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 |
| Kd | adsorption ratio |
| kg | kilogram |
| kkg | metric ton |
| $K_{oc}$ | organic carbon partition coefficient |
| $K_{ow}$ | octanol-water partition coefficient |
| L | liter |
| LC | liquid chromatography |
| $LC_{50}$ | lethal concentration, 50% kill |
| $LC_{Lo}$ | lethal concentration, low |
| $LD_{50}$ | lethal dose, 50% kill |
| $LD_{Lo}$ | lethal dose, low |
| LDH | lactic dehydrogenase |
| LH | luteinizing hormone |
| LOAEL | lowest-observed-adverse-effect level |
| LSE | Levels of Significant Exposure |
| $LT_{50}$ | lethal time, 50% kill |
| m | meter |
| MA | *trans,trans*-muconic acid |

| | |
|---|---|
| MAL | maximum allowable level |
| mCi | millicurie |
| 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 |
| 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 |

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

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# APPENDIX D. INDEX