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Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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ABBREVIATIONS

AAP	Army ammunition plant
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	acetylcholinesterase
ADAF	age-dependent adjustment factor
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AOP	adverse outcome pathway
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BDNF	brain-derived neurotrophic factor
BHC	beta-hexachlorocyclohexane
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMDU	benchmark dose upper bound
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CAAC	Chemical Assessment Advisory Committee
CASRN	Chemical Abstracts Service Registry Number
CCL	Contaminant Candidate List
CI	confidence interval
CICAD	Concise International Chemical Assessment Document
CNS	central nervous system
CSF	cerebrospinal fluid
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DDT	dichlorodiphenyltrichloroethane
d.f.	degrees of freedom
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNX	1-nitro-3,5-dinitroso-1,3,5-triazacyclohexane
DTIC	Defense Technical Information Center
EEG	electroencephalogram
EHC	Environmental Health Criteria
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FOB	functional observational battery
FUDS	Formerly Used Defense Sites
GABA	gamma-amino butyric acid
GD	gestational day
GI	gastrointestinal
GLP	good laboratory practices
HED	human equivalent dose
HERO	Health and Environmental Research Online
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
miRNA	microRNA
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
MOA	mode of action
MRL	Minimal Risk Level
NAPDH	nicotinamide adenine dinucleotide phosphate
NAS	National Academy of Science
NCE	normochromatic erythrocyte
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
NHANES	National Health and Nutrition Examination Survey
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NPL	National Priorities List
NRC	Nuclear Regulatory Commission
NSCEP	National Service Center for Environmental Publications

NTP	National Toxicology Program
NZW	New Zealand White
OR	odds ratio
ORD	Office of Research and Development
OSF	oral slope factor
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCE	polychromatic erythrocyte
PEL	Permissible Exposure Limit
PND	postnatal day
POD	point of departure
PWG	Pathology Working Group
RBC	red blood cell
RDX	Royal Demolition eXplosive (hexahydro-1,3,5-trinitro-1,3,5-triazine)
REL	Recommended Exposure Limit
RfC	inhalation reference concentration
RfD	oral reference dose
SDMS	spontaneous death or moribund sacrifice
SDWA	Safe Drinking Water Act
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SLE	systemic lupus erythematosus
SS	scheduled sacrifice
TLV	Threshold Limit Value
TNT	trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
U.S.	United States of America
UCM	Unregulated Contaminant Monitoring
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertain factor
UF_S	subchronic-to-chronic uncertainty factor
WBC	white blood cell
WHO	World Health Organization

AUTHORS | CONTRIBUTORS | REVIEWERS

Assessment Team

Louis D'Amico, Ph.D. (Assessment Manager) Todd Blessinger, Ph.D. Ravi Subramaniam, Ph.D.	U.S. EPA/Office of Research and Development/National Center for Environmental Assessment
Christopher Brinkerhoff, Ph.D.	Former ORISE Postdoctoral Fellow at U.S. EPA/ORD/NCEA Currently at U.S. EPA/Office of Chemical Safety and Pollution Prevention

Contributors

Rob DeWoskin, Ph.D. (retired) Belinda Hawkins, Ph.D. Karen Hogan, MS Andrew Kraft, Ph.D. Jordan Trecki, Ph.D. (formerly with EPA) Scott Wesselkamper, Ph.D.	U.S. EPA/ORD/NCEA
Tammy Stoker, Ph.D. Charles Wood, DVM, PhD, DACVP	U.S. EPA/ORD/National Health and Environmental Research Laboratory (NHEERL)
Anne Loccisano, Ph.D.	Former ORISE Postdoctoral Fellow at U.S. EPA/ORD/NCEA
Kelly Garcia Carolyn Gigot	EPA Environmental Health Assessment Support Associate (ORAU Student Services Contractor)

Production Team

Maureen Johnson Vicki Soto Dahnish Shams	U.S. EPA/ORD/NCEA

Contractor Support

Heather Carlson-Lynch, S.M., DABT Julie Melia, Ph.D., DABT Megan Riccardi, M.S.	SRC, Inc., North Syracuse, NY
Pam Ross, M.S. Robin Blain, Ph.D.	ICF International, Fairfax, VA

Executive Direction

Tina Bahadori, Sc.D. (Center Director, National Program Director, HHRA) Kenneth Olden, Ph.D., Sc.D., L.H.D. (Center Director - Retired) Michael Slimak, Ph.D. (Acting Center Director)	U.S. EPA/ORD/NCEA

John Vandenberg, Ph.D, (Former National Program Director, HHRA)
Lynn Flowers, Ph.D., DABT (Former Associate Director for Health)
Kristina Thayer, Ph.D. (IRIS Division Director)
Vincent Cogliano, Ph.D. (Former IRIS Division Director)
James Avery, Ph.D. (IRIS Division Deputy Director)
Gina Perovich, M.S. (Former IRIS Division Deputy Director)
Samantha Jones, Ph.D. (IRIS Associate Director for Science)
Susan Rieth, MPH (Quantitative Modeling Branch Chief)

Internal Review Team

General Toxicity/Immunotoxicity/Cancer Workgroup — U.S. EPA/ORD/NCEA
Epidemiology Workgroup
Neurotoxicity Workgroup
Pharmacokinetics Workgroup
Reproductive and Developmental Toxicity Workgroup
Scoping and Problem Formulation Workgroup
Statistics Workgroup
Toxicity Pathways Workgroup

Reviewers

This assessment was provided for review to scientists in EPA's program and regional offices. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection
Office of Chemical Safety and Pollution Prevention/Office of Pesticide Programs
Office of Land and Emergency Management
Office of Land and Emergency Management/Federal Facilities Forum
Office of Water
Region 2, New York City
Region 8, Denver

This assessment was provided for review to other federal agencies and the Executive Office of the President (EOP). A summary and EPA's disposition of major comments from the other federal agencies and the EOP is available on the IRIS website. Comments were submitted by:

Department of Defense
Department of Energy
Department of Health and Human Services/Agency for Toxic Substances and Disease Registry
Department of Health and Human Services/National Institute of Environmental Health Sciences/National Toxicology Program
Department of Health and Human Services/National Institute for Occupational Safety and Health
National Aeronautics and Space Administration
Executive Office of the President/Council on Environmental Quality
Executive Office of the President/Office of Management and Budget

This assessment was released for public comment on March 10, 2016 and comments were due on May 9, 2016. The public comments are available on Regulations.gov. A summary and EPA's disposition of the comments from the public is included in the external review draft assessment on the IRIS website. Comments were received from the following entities:

Nancy Beck, American Chemistry Council (currently Deputy Assistant Administrator, Office of Chemical Safety and Pollution Prevention, U.S. EPA)
Andy Nong, Health Canada
Johns Hopkins Bloomberg School of Public Health, Special Studies in Risk Assessment Class as submitted by Mary Fox
Ron Melnick
U.S. Army Public Health Command and Uniformed Services University of the Health Sciences
Larry Williams
Anonymous member of the public

A public science meeting was held on May 10, 2016 to obtain public input on the IRIS Toxicological Review of RDX (Public Comment Draft). Public commenters, stakeholders, and members of the scientific community were joined by independent experts identified by the National Academies' National Research Council (NRC) (identified by * below) in a discussion of key science topics. Discussants and public commenters were:

Desmond Bannon	U.S. Army Public Health Center
Nancy Beck	American Chemistry Council (currently Deputy Assistant Administrator, Office of Chemical Safety and Pollution Prevention, U.S. EPA)
Maria Braga*	Uniformed Services University of the Health Sciences
Marion Ehrich*	Virginia-Maryland College of Veterinary Medicine (Virginia Tech)
Kendall Frazier*	GlaxoSmithKline
Andy Nong*	Health Canada
Karen Regan*	Research Pathology Associates
Evelyn Tiffany-Castiglioni*	Texas A&M University
Larry Williams	

This assessment was peer reviewed by independent, expert scientists external to EPA convened by EPA's Science Advisory Board (SAB), the SAB Chemical Assessment Advisory Committee (CAAC) Augmented for Review of the Draft IRIS RDX Assessment. A peer-review meeting was held on December 12–14, 2016. The report of the SAB's review of the EPA's draft Toxicological Review of RDX, dated September 27, 2017, is available on the IRIS website. A summary and EPA's disposition of the comments received from the SAB is included in Appendix E.

Dr. Kenneth Ramos (Chair)	Associate Vice-President of Precision Health Sciences and Professor of Medicine, University of Arizona Health Sciences, Tucson, AZ
Dr. Hugh A. Barton	Associate Research Fellow, Pharmacokinetics, Dynamics, and Metabolism, Pfizer Inc., Groton, CT
Dr. Maarten C. Bosland	Professor of Pathology, College of Medicine, University of Illinois at Chicago, Chicago, IL
Dr. Mary Boudreau	Research Toxicologist, Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR
Dr. James V. Bruckner	Professor, Department of Pharmacology & Toxicology, College of Pharmacy, University of Georgia, Athens, GA
Dr. George Cobb	Professor, Environmental Science, College of Arts and Sciences, Baylor University, Waco, TX
Dr. David Eastmond	Professor and Chair, Department of Cell Biology and Neuroscience, Toxicology Graduate Program, University of California at Riverside, Riverside, CA
Dr. Joanne English	Independent Consultant, Menlo Park, CA
Dr. Alan Hoberman	Toxicologist, Research, Charles River Laboratories, Inc., Horsham, PA
Dr. Jacqueline Hughes-Oliver	Professor, Statistics Department, North Carolina State University, Raleigh, NC
Dr. Susan Laffan	Safety Assessment, GlaxoSmithKline, King of Prussia, PA

Dr. Lawrence Lash	Professor, Department of Pharmacology, Wayne State University School of Medicine, Wayne State University, Detroit, MI
Dr. Stephen Lasley	Professor of Pharmacology and Assistant Head, Cancer Biology & Pharmacology, College of Medicine, University of Illinois at Chicago, Peoria, IL
Dr. Melanie Marty	Adjunct Professor, Environmental Toxicology, University of California at Davis, Davis, CA
Dr. Marvin Meistrich	Professor, Experimental Radiation Oncology, M.D. Anderson Cancer Center, University of Texas, Houston, TX
Dr. Marilyn Morris	Professor of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY
Dr. Victoria Persky	Professor, Epidemiology & Biostatistics Program, School of Public Health, University of Illinois at Chicago, Chicago, IL
Dr. Isaac Pessah	Professor, Molecular Biosciences, School of Veterinary Medicine, University of California at Davis, Davis, CA
Dr. Kenneth M. Portier	Independent Consultant, Athens, GA
Dr. Samba Reddy	Professor, Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M University, Bryan, TX
Dr. Stephen M. Roberts	Professor, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL
Dr. Thomas Rosol	Professor, Veterinary Biosciences, College of Veterinary Medicine, Ohio State University, Columbus, OH
Dr. Alan Stern	Chief, Bureau for Risk Analysis, Division of Science, Research and Environmental Health, New Jersey Department of Environmental Protection, Trenton, NJ
Dr. Robert Turesky	Professor, Masonic Cancer Center and Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN

PREFACE

This Toxicological Review critically reviews the publicly available studies on hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, Royal Demolition eXplosive, or cyclonite) in order to identify its adverse health effects and to characterize exposure-response relationships. It was prepared under the auspices of the U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) Program. This assessment updates a previous IRIS assessment of RDX that included an oral reference dose (RfD) for effects other than cancer (posted in 1988), a determination on the carcinogenicity of RDX, and derivation of an oral slope factor (OSF) to quantify the cancer risk associated with RDX exposure (posted in 1990). New information has become available, and this assessment reviews information on all health effects by all exposure routes.

A public meeting was held in December 2013 to obtain input on preliminary materials for RDX, including draft literature searches and associated search strategies, evidence tables, and exposure-response arrays prior to the development of the IRIS assessment. All public comments provided on the preliminary materials were taken into consideration in developing the draft assessment. A second public meeting was held in May 2016 to discuss key science topics on the public comment draft assessment. These topics included: (1) suppurative prostatitis as a marker for hazard to the urogenital system following RDX exposure; (2) evaluation and use of RDX physiologically-based pharmacokinetic (PBPK) models; (3) neurotoxicity observed with RDX and consideration of dose and duration of exposure and the potential relationship to mortality; and (4) other science topics in the RDX assessment. Independent experts identified by the National Academies' National Research Council (NRC) joined members of the scientific community, stakeholders, and the general public in the discussion of these science topics. The complete set of public comments submitted in connection with the December 2013 and May 2016 public meetings are available on the docket at https://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-0430).

Organ/system-specific reference values are calculated based on nervous system, urinary system (kidney and bladder), and prostate. These reference values may be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system.

This assessment was conducted in accordance with EPA guidance, which is summarized in the Preamble to IRIS Toxicological Reviews and cited at appropriate places in this assessment. The findings of this assessment and related documents produced during its development are available on the IRIS website (https://www.epa.gov/iris). Appendices containing information on assessments by other health agencies, details of the literature search strategy, toxicokinetic

information, summaries of supplementary toxicity information, and dose-response modeling are provided as Supplemental Information to this assessment (see Appendices A to D).

The IRIS Program released preliminary assessment materials for RDX in December 2013 and the draft assessment for public comment in March 2016, during the period of development and implementation of systematic review methods by the IRIS Program. The approach to implementation is to use procedures and tools available at the time, without holding assessments until new methods become available. Accordingly, the IRIS Program conducted literature searches and evaluated studies using tools and documentation standards then available. Updated problem formulation materials and systematic review protocol development began with assessments started in 2015, after this assessment was well into assessment development. Implementation of systematic review is a process of continuous improvement and this assessment represents a step in the evolution of the IRIS Program.

Uses and Environmental Occurrence

RDX is a military munitions explosive with limited civilian commercial uses (Gadagbui et al., 2012). In the United States, RDX is produced at Army ammunition plants (AAPs) and is not manufactured commercially. RDX production peaked in the 1960s; 180 million pounds per year were produced from 1969 to 1971. Yearly total production dropped to 16 million pounds in 1984 (ATSDR, 2012). According to the U.S. EPA ChemView Tool (https://chemview.epa.gov/chemview), the aggregate national production volume in 2015 was between 1 million and 10 million pounds.

RDX can be released into environmental media (air, water, soil) as a result of waste generated during manufacture, packing, or disposal of the pure product, or use and disposal of RDX-containing munitions (ATSDR, 2012; Gadagbui et al., 2012; ATSDR, 1999, 1993, 1992). RDX is mobile in soil; leaching into groundwater has been reported in samples from military facilities (Best et al., 1999a; Godejohann et al., 1998; Bart et al., 1997; Steuckart et al., 1994; Spanggord et al., 1980). RDX transport in soil is generally through dissolution by precipitation and subsequent downward movement, including migration to groundwater aquifers, and not much via surface runoff (U.S. EPA, 2012b). Discussion of RDX properties and fate and transport is available in U.S. EPA (2012b) and on the EPA's Chemistry Dashboard at https://comptox.epa.gov/dashboard/. Detectable levels of RDX have been observed in plants irrigated or grown with RDX-contaminated water (Best et al., 1999b; Simini and Checkai, 1996; Harvey et al., 1991). RDX has also been detected in indoor air samples from military facilities where RDX is produced (Bishop et al., 1988).

Exposures to RDX among the general population are likely to be confined to individuals in or around active or formerly-used military facilities where RDX is or was produced, stored, or used. Oral, inhalation, and dermal routes of exposure may be relevant.

As of 2018, RDX was detected in surface water, groundwater, sediment, or soil at 32 active U.S. EPA National Priorities List (NPL) sites. The NPL serves as a list of sites with known or threatened releases of hazardous substances, pollutants, or contaminants throughout the United States and its territories. The NPL aids the Agency in identifying the most serious sites that may

warrant cleanup. The majority of the NPL sites where RDX was listed are associated with military facilities. Based on Department of Defense records, Gadagbui et al. (2012) reported that RDX contamination is present on 76 active military sites, 9 closed sites, and 15 sites under the Formerly Used Defense Sites (FUDS) program. Not all sites under the FUDS program have been sampled, and additional sites with RDX contamination in this program could be identified.

As of 2018, RDX was not regulated under the Safe Drinking Water Act (SDWA), although it was included as a contaminant to be monitored under the Unregulated Contaminant Monitoring (UCM) Rule by EPA's Office of Water from 2007 to 2011. Contaminants included in the UCM program are suspected of being present in drinking water, but do not have existing health-based standards set under the SDWA. RDX has also been included on the Office of Water's Drinking Water Contaminant Candidate List (CCL) since the initial listing was published in 1998. The presence of a chemical on the list suggests that it is known or anticipated to occur in public water systems.

Assessments by Other National and International Health Agencies

Toxicity information on RDX has been evaluated by the Agency for Toxic Substances and Disease Registry (ATSDR), National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA), and Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS). The results of these assessments (as of 2018) are presented in Appendix A of the Supplemental Information. It is important to recognize that the assessments performed by other health agencies may have been prepared for different purposes and may utilize different methods. In addition, newer studies may be included in the IRIS assessment.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), or hotline.iris@epa.gov.

PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

The summarizes the objectives and scope of the IRIS program, general principles and systematic review procedures used in developing IRIS assessments, and the overall development process and document structure.

Objectives and Scope of the IRIS Program

Soon after EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in support of actions to protect human health and the environment. EPA's IRIS program[1] contributes to this endeavor by reviewing epidemiologic and experimental studies of chemicals in the environment to identify adverse health effects and characterize exposure–response relationships. Health agencies worldwide use IRIS assessments, which are also a scientific resource for researchers and the public.

IRIS assessments cover the hazard identification and dose–response steps of risk assessment. Exposure assessment and risk characterization are outside the scope of IRIS assessments, as are political, economic, and technical aspects of risk management. An IRIS assessment may cover one chemical, a group of structurally or toxicologically related chemicals, or a chemical mixture. Exceptions outside the scope of the IRIS program are radionuclides, chemicals used only as pesticides, and the "criteria air pollutants" (particulate matter, ground-level ozone, carbon monoxide, sulfur oxides, nitrogen oxides, and lead).

Enhancements to the IRIS program are improving its science, transparency, and productivity. To improve the science, the IRIS program is adapting and implementing principles of systematic review (i.e., using explicit methods to identify, evaluate, and synthesize study findings). To increase transparency, the IRIS program discusses key science issues with the scientific community and the public as it begins an assessment. External peer review, independently managed and in public, improves both science and transparency. Increased productivity requires that assessments be concise, focused on EPA's needs, and completed without undue delay.

IRIS assessments follow EPA guidance[2] and standardized practices of systematic review. This Preamble summarizes and does not change IRIS operating procedures or EPA guidance.

Periodically, the IRIS program asks for nomination of agents for future assessment or reassessment. Selection depends on EPA's priorities, relevance to public health, and availability of pertinent studies. The IRIS multiyear agenda[3] lists upcoming assessments. The IRIS program may also assess other agents in anticipation of public health needs.

Planning an Assessment: Scoping, Problem Formulation, and Protocols

Early attention to planning ensures that IRIS assessments meet their objectives and properly frame science issues.

Scoping refers to the first step of planning, where the IRIS program consults with EPA's program and regional offices to ascertain their

[1]IRIS program website: http://www.epa.gov/iris/.
[2]EPA guidance documents: http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance/.
[3]IRIS multiyear agenda: https://www.epa.gov/iris/iris-agenda.

needs. Scoping specifies the agents an assessment will address, routes and durations of exposure, susceptible populations and lifestages, and other topics of interest.

Problem formulation refers to the science issues an assessment will address and includes input from the scientific community and the public. A preliminary literature survey, beginning with secondary sources (e.g., assessments by national and international health agencies and comprehensive review articles), identifies potential health outcomes and science issues. It also identifies related chemicals (e.g., toxicologically active metabolites and compounds that metabolize to the chemical of interest).

Each IRIS assessment comprises multiple systematic reviews for multiple health outcomes. It also evaluates hypothesized mechanistic pathways and characterizes exposure–response relationships. An assessment may focus on important health outcomes and analyses rather than expand beyond what is necessary to meet its objectives.

Protocols refer to the systematic review procedures planned for use in an assessment. They include strategies for literature searches, criteria for study inclusion or exclusion, considerations for evaluating study methods and quality, and approaches to extracting data. Protocols may evolve as an assessment progresses and new agent-specific insights and issues emerge.

Identifying and Selecting Pertinent Studies

IRIS assessments conduct systematic literature searches with criteria for inclusion and exclusion. The objective is to retrieve the pertinent primary studies (i.e., studies with original data on health outcomes or their mechanisms). *PECO statements* (Populations, Exposures, Comparisons, Outcomes) govern the literature searches and screening criteria. "Populations" and animal species generally have no restrictions. "Exposures" refers to the agent and related chemicals identified during scoping and problem formulation and may consider route, duration, or timing of exposure. "Comparisons" means studies that allow comparison of effects across different levels of exposure. "Outcomes" may become more specific (e.g., from "toxicity" to "developmental toxicity" to "hypospadias") as an assessment progresses.

For studies of absorption, distribution, metabolism, and elimination, the first objective is to create an inventory of pertinent studies. Subsequent sorting and analysis facilitates characterization and quantification of these processes.

Studies on mechanistic events can be numerous and diverse. Here, too, the objective is to create an inventory of studies for later sorting to support analyses of related data. The inventory also facilitates generation and evaluation of hypothesized mechanistic pathways.

The IRIS program posts initial protocols for literature searches on its website and adds search results to EPA's HERO database.[4] Then the IRIS program takes extra steps to ensure identification of pertinent studies: by encouraging the scientific community and the public to identify additional studies and ongoing research; by searching for data submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act; and by considering late-breaking studies that would impact the credibility of the conclusions, even during the review process.[5]

Evaluating Study Methods and Quality

IRIS assessments evaluate study methods and quality, using uniform approaches for each group of similar studies. The objective is that subsequent syntheses can weigh study results on their merits. Key concerns are potential *bias* (factors that affect the magnitude or direction of an effect) and *insensitivity* (factors that limit the ability of a study to detect a true effect).

[4]Health and Environmental Research Online: https://hero.epa.gov/hero/.
[5]IRIS "stopping rules": https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf.

For human and animal studies, the evaluation of study methods and quality considers study design, exposure measures, outcome measures, data analysis, selective reporting, and study sensitivity. For human studies, this evaluation also considers selection of participant and referent groups and potential confounding. Emphasis is on discerning bias that could substantively change an effect estimate, considering also the expected direction of the bias. Low sensitivity is a bias towards the null.

Study-evaluation considerations are specific to each study design, health effect, and agent. Subject-matter experts evaluate each group of studies to identify characteristics that bear on the informativeness of the results. For carcinogenicity, neurotoxicity, reproductive toxicity, and developmental toxicity, there is EPA guidance for study evaluation (U.S. EPA, 2005a, 1998, 1996, 1991). As subject-matter experts examine a group of studies, additional agent-specific knowledge or methodologic concerns may emerge and a second pass become necessary.

Assessments use evidence tables to summarize the design and results of pertinent studies. If tables become too numerous or unwieldy, they may focus on effects that are more important or studies that are more informative.

The IRIS program posts initial protocols for study evaluation on its website, then considers public input as it completes this step.

Integrating the Evidence of Causation for Each Health Outcome

Synthesis within lines of evidence. For each health outcome, IRIS assessments synthesize the human evidence and the animal evidence, augmenting each with informative subsets of mechanistic data. Each synthesis considers aspects of an association that may suggest causation: consistency, exposure–response relationship, strength of association, temporal relationship, biological plausibility, coherence, and "natural experiments" in humans (U.S. EPA, 1994, §2.1.3) (U.S. EPA, 2005a, §2.5).

Each synthesis seeks to reconcile ostensible inconsistencies between studies, taking into account differences in study methods and quality. This leads to a distinction between *conflicting evidence* (unexplained positive and negative results in similarly exposed human populations or in similar animal models) and *differing results* (mixed results attributable to differences between human populations, animal models, or exposure conditions) (U.S. EPA, 2005a, §2.5).

Each synthesis of human evidence explores alternative explanations (e.g., chance, bias, or confounding) and determines whether they may satisfactorily explain the results. Each synthesis of animal evidence explores the potential for analogous results in humans. Coherent results across multiple species increase confidence that the animal results are relevant to humans.

Mechanistic data are useful to augment the human or animal evidence with information on precursor events, to evaluate the human relevance of animal results, or to identify susceptible populations and lifestages. An agent may operate through multiple mechanistic pathways, even if one hypothesis dominates the literature (U.S. EPA, 2005a, §2.4.3.3).

Integration across lines of evidence. For each health outcome, IRIS assessments integrate the human, animal, and mechanistic evidence to answer the question: *What is the nature of the association between exposure to the agent and the health outcome?*

For cancer, EPA includes a standardized hazard descriptor in characterizing the strength of the evidence of causation. The objective is to promote clarity and consistency of conclusions across assessments (U.S. EPA, 2005a, §2.5).

Carcinogenic to humans: convincing epidemiologic evidence of a causal association; or strong human evidence of cancer or its key precursors, extensive animal evidence, identification of mode-of-action and its key precursors in animals, and strong evidence that they are anticipated in humans.

Likely to be carcinogenic to humans: evidence that demonstrates a potential hazard to humans. Examples include a plausible association in humans with supporting experimental evidence, multiple positive

results in animals, a rare animal response, or a positive study strengthened by other lines of evidence.

Suggestive evidence of carcinogenic potential: evidence that raises a concern for humans. Examples include a positive result in the only study, or a single positive result in an extensive database.

Inadequate information to assess carcinogenic potential: no other descriptors apply. Examples include little or no pertinent information, *conflicting evidence*, or negative results not sufficiently robust for *not likely*.

Not likely to be carcinogenic to humans: robust evidence to conclude that there is no basis for concern. Examples include no effects in well-conducted studies in both sexes of multiple animal species, extensive evidence showing that effects in animals arise through modes-of-action that do not operate in humans, or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

If there is credible evidence of carcinogenicity, there is an evaluation of mutagenicity, because this influences the approach to dose–response assessment and subsequent application of adjustment factors for exposures early in life (U.S. EPA, 2005a, §3.3.1, §3.5), (U.S. EPA, 2005b, §5).

Selecting Studies for Derivation of Toxicity Values

The purpose of toxicity values (slope factors, unit risks, reference doses, reference concentrations; see section 7) is to estimate exposure levels likely to be without appreciable risk of adverse health effects. EPA uses these values to support its actions to protect human health.

The health outcomes considered for derivation of toxicity values may depend on the hazard descriptors. For example, IRIS assessments generally derive cancer values for agents that are *carcinogenic* or *likely to be carcinogenic*, and sometimes for agents with *suggestive evidence* (U.S. EPA, 2005a, §3).

Derivation of toxicity values begins with a new evaluation of studies, as some studies used qualitatively for hazard identification may not be useful quantitatively for exposure–response assessment. Quantitative analyses require quantitative measures of exposure and response. An assessment weighs the merits of the human and animal studies, of various animal models, and of different routes and durations of exposure (U.S. EPA, 1994, §2.1). Study selection is not reducible to a formula, and each assessment explains its approach.

Other biological determinants of study quality include appropriate measures of exposure and response, investigation of early effects that precede overt toxicity, and appropriate reporting of related effects (e.g., combining effects that comprise a syndrome, or benign and malignant tumors in a specific tissue).

Statistical determinants of study quality include multiple levels of exposure (to characterize the shape of the exposure–response curve) and adequate exposure range and sample sizes (to minimize extrapolation and maximize precision) (U.S. EPA, 2012, §2.1).

Studies of low sensitivity may be less useful if they fail to detect a true effect or yield toxicity values with wide confidence limits.

Deriving Toxicity Values

General approach. EPA guidance describes a two-step approach to dose–response assessment: analysis in the range of observation, then extrapolation to lower levels. Each toxicity value pertains to a route (e.g., oral, inhalation, dermal) and duration or timing of exposure (e.g., chronic, subchronic, gestational) (U.S. EPA, 2002, §4).

IRIS assessments derive a candidate value from each suitable data set. Consideration of candidate values yields a toxicity value for each organ or system. Consideration of the organ/system-specific values results in the selection of an overall toxicity value to cover all health outcomes. The organ/system-specific values are useful for subsequent cumulative risk assessments that consider the combined effect of

multiple agents acting at a common anatomical site.

Analysis in the range of observation. Within the observed range, the preferred approach is modeling to incorporate a wide range of data. Toxicokinetic modeling has become increasingly common for its ability to support target-dose estimation, cross-species adjustment, or exposure-route conversion. If data are too limited to support toxicokinetic modeling, there are standardized approaches to estimate daily exposures and scale them from animals to humans (U.S. EPA, 1994, §3), (U.S. EPA, 2005a, §3.1), (U.S. EPA, 2011, 2006).

For human studies, an assessment may develop exposure–response models that reflect the structure of the available data (U.S. EPA, 2005a, §3.2.1). For animal studies, EPA has developed a set of empirical ("curve-fitting") models[6] that can fit typical data sets (U.S. EPA, 2005a, §3.2.2). Such modeling yields a *point of departure*, defined as a dose near the lower end of the observed range, without significant extrapolation to lower levels (e.g., the estimated dose associated with an extra risk of 10% for animal data or 1% for human data, or their 95% lower confidence limits)(U.S. EPA, 2005a, §3.2.4), (U.S. EPA, 2012, §2.2.1).

When justified by the scope of the assessment, toxicodynamic ("biologically based") modeling is possible if data are sufficient to ascertain the key events of a mode-of-action and to estimate their parameters. Analysis of model uncertainty can determine the range of lower doses where data support further use of the model (U.S. EPA, 2005a, §3.2.2, §3.3.2).

For a group of agents that act at a common site or through common mechanisms, an assessment may derive relative potency factors based on relative toxicity, rates of absorption or metabolism, quantitative structure–activity relationships, or receptor-binding characteristics (U.S. EPA, 2005a, §3.2.6).

Extrapolation: slope factors and unit risks. An *oral slope factor* or an *inhalation unit risk* facilitates subsequent estimation of human cancer risks. Extrapolation proceeds linearly (i.e., risk proportional to dose) from the point of departure to the levels of interest. This is appropriate for agents with direct mutagenic activity. It is also the default if there is no established mode-of-action (U.S. EPA, 2005a, §3.3.1, §3.3.3).

Differences in susceptibility may warrant derivation of multiple slope factors or unit risks. For early-life exposure to carcinogens with a mutagenic mode-of-action, EPA has developed default *age-dependent adjustment factors* for agents without chemical-specific susceptibility data (U.S. EPA, 2005a, §3.5), (U.S. EPA, 2005b, §5).

If data are sufficient to ascertain the mode-of-action and to conclude that it is not linear at low levels, extrapolation may use the reference-value approach (U.S. EPA, 2005a, §3.3.4).

Extrapolation: reference values. An *oral reference dose* or an *inhalation reference concentration* is an estimate of human exposure (including in susceptible populations) likely to be without appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002, §4.2). Reference values generally cover effects other than cancer. They are also appropriate for carcinogens with a nonlinear mode-of-action.

Calculation of reference values involves dividing the point of departure by a set of *uncertainty factors* (each typically 1, 3, or 10, unless there are adequate chemical-specific data) to account for different sources of uncertainty and variability (U.S. EPA, 2002, §4.4.5), (U.S. EPA, 2014).

Human variation: An uncertainty factor covers susceptible populations and lifestages that may respond at lower levels, unless the data originate from a susceptible study population.

Animal-to-human extrapolation: For reference values based on animal results, an uncertainty factor reflects cross-species differences, which may cause humans to respond at lower levels.

Subchronic-to-chronic exposure: For chronic reference values based on subchronic studies, an uncertainty factor reflects the likelihood that a lower level over a longer duration may induce a similar response. This

[6]Benchmark Dose Software: http://www.epa.gov/bmds/.

factor may not be necessary for reference values of shorter duration.

Adverse-effect level to no-observed-adverse-effect level: For reference values based on a lowest-observed-adverse-effect level, an uncertainty factor reflects a level judged to have no observable adverse effects.

Database deficiencies: If there is concern that future studies may identify a more sensitive effect, target organ, population, or lifestage, a *database uncertainty factor* reflects the nature of the database deficiency.

Process for Developing and Peer-Reviewing IRIS Assessments

The IRIS process (revised in 2009 and enhanced in 2013) involves extensive public engagement and multiple levels of scientific review and comment. IRIS program scientists consider all comments. Materials released, comments received from outside EPA, and disposition of major comments (steps 3, 4, and 6 below) become part of the public record.

Step 1: Draft development. As outlined in section 2 of this Preamble, IRIS program scientists specify the scope of an assessment and formulate science issues for discussion with the scientific community and the public. Next, they release initial protocols for the systematic review procedures planned for use in the assessment. IRIS program scientists then develop a first draft, using structured approaches to identify pertinent studies, evaluate study methods and quality, integrate the evidence of causation for each health outcome, select studies for derivation of toxicity values, and derive toxicity values, as outlined in Preamble sections 3–7.

Step 2: Agency review. Health scientists across EPA review the draft assessment.

Step 3: Interagency science consultation. Other federal agencies and the Executive Office of the President review the draft assessment.

Step 4: Public comment, followed by external peer review. The public reviews the draft assessment. IRIS program scientists release a revised draft for independent external peer review. The peer reviewers consider whether the draft assessment assembled and evaluated the evidence according to EPA guidance and whether the evidence justifies the conclusions.

Step 5: Revise assessment. IRIS program scientists revise the assessment to address the comments from the peer review.

Step 6: Final agency review and interagency science discussion. The IRIS program discusses the revised assessment with EPA's program and regional offices and with other federal agencies and the Executive Office of the President.

Step 7: Post final assessment. The IRIS program posts the completed assessment and a summary on its website.

General Structure of IRIS Assessments

Main text. IRIS assessments generally comprise two major sections: (1) Hazard Identification and (2) Dose–Response Assessment. Section 1.1 briefly reviews chemical properties and toxicokinetics to describe the disposition of the agent in the body. This section identifies related chemicals and summarizes their health outcomes, citing authoritative reviews. If an assessment covers a chemical mixture, this section discusses environmental processes that alter the mixtures humans encounter and compares them to mixtures studied experimentally.

Section 1.2 includes a subsection for each major health outcome. Each subsection discusses the respective literature searches and study considerations, as outlined in Preamble sections 3 and 4, unless covered in the front matter. Each subsection concludes with evidence synthesis and integration, as outlined in Preamble section 5.

Section 1.3 links health hazard information to dose–response analyses for each health outcome. One subsection identifies susceptible populations and lifestages, as observed in human or animal studies or inferred from mechanistic

data. These may warrant further analysis to quantify differences in susceptibility. Another subsection identifies biological considerations for selecting health outcomes, studies, or data sets, as outlined in Preamble section 6.

Section 2 includes a subsection for each toxicity value. Each subsection discusses study selection, methods of analysis, and derivation of a toxicity value, as outlined in Preamble sections 6 and 7.

Front matter. The Executive Summary provides information historically included in IRIS summaries on the IRIS program website. Its structure reflects the needs and expectations of EPA's program and regional offices.

A section on systematic review methods summarizes key elements of the protocols, including methods to identify and evaluate pertinent studies. The final protocols appear as an appendix.

The Preface specifies the scope of an assessment and its relation to prior assessments. It discusses issues that arose during assessment development and emerging areas of concern.

This Preamble summarizes general procedures for assessments begun after the date below. The Preface identifies assessment-specific approaches that differ from these general procedures.

August 2016

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a synthetic chemical used primarily as a military explosive. RDX releases have been reported in air, water, and soil. Exposure to RDX is likely limited to individuals in or around military facilities where RDX is or was produced, used, or stored. Oral exposure may occur from drinking contaminated groundwater or ingesting crops irrigated with contaminated water. Inhalation or dermal exposures are more likely in occupational settings.

Epidemiological studies provide only limited information on worker populations exposed to RDX; several case reports describe effects primarily in the nervous system following acute exposure to RDX. Animal studies of ingested RDX demonstrate toxicity, including effects on the nervous system, urinary system (kidney and bladder), and prostate.

Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on evidence of positive trends in liver and lung tumor incidence in experimental animals. There are no data on the carcinogenicity of RDX in humans.

ES.1 EVIDENCE FOR HAZARDS OTHER THAN CANCER: ORAL EXPOSURE

Nervous system effects are a human hazard of RDX exposure. Several human case reports and animal studies provide consistent evidence of an association between RDX exposure and effects on the nervous system, including findings related to increased seizure induction, including abnormal electrical activity, convulsions, tremors, and reducing the threshold for seizure induction by other stimuli; behavioral effects that may be related to seizures such as hyperirritability, hyper-reactivity, and other behavioral changes. Mechanistic data support the hypothesis that RDX-induced seizures and related behavioral effects likely result from inhibition of GABAergic signaling in the limbic system. Some investigators reported that unscheduled deaths in experimental animals exposed to RDX were frequently preceded by convulsions or seizures.

Urinary system effects are a potential human hazard of RDX exposure based largely on observations of histopathological changes in the kidney and urinary bladder of male rats exposed to RDX at doses higher than those associated with nervous system effects. The available evidence indicates that male rats are more sensitive than females, and rats are more sensitive than mice to RDX-related urinary system toxicity. There is suggestive evidence of male prostate effects associated with RDX exposure based on an increased incidence of suppurative prostatitis in male rats exposed to RDX in the diet for 2 years, in one of the few studies that evaluated the prostate. There is no known mode of action (MOA) for effects of RDX exposure on the urinary system or prostate, although there are studies indicating GABA helps regulate urinary system and prostate

function. Evidence for effects on other organs/systems, or developmental effects, was more limited than for the endpoints summarized above.

E.S.1.1 Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ-specific RfDs were derived for hazards associated with RDX exposure (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Table ES-1. Organ/system-specific RfDs and overall RfD for RDX

Effect	Basis	RfD (mg/kg-d)	Study exposure description	Confidence
Nervous system	Convulsions	4×10^{-3}	Subchronic	Medium
Urinary system	Kidney medullary papillary necrosis	1×10^{-2}	Chronic	Medium
Prostate	Suppurative prostatitis	8×10^{-4}	Chronic	Low
Overall RfD	**Nervous system effects**	**4×10^{-3}**	**Subchronic**	**Medium**

The overall RfD (see Table ES-2) is derived to be protective of all types of hazards associated with RDX exposure. Although the RfD for prostate effects results in a smaller value, it was not selected as the overall RfD due to uncertainties in the evaluation of this endpoint ("low confidence"). The effect of RDX on the nervous system was chosen as the basis for the overall RfD because nervous system effects were observed most consistently across studies, species, and exposure durations, and because they represent a sensitive human hazard of RDX exposure. Evidence for effects of RDX on the urinary system and prostate is more limited relative to the effects of RDX on the nervous system. Incidence of seizures or convulsions as reported in a subchronic gavage study (Crouse et al., 2006) was selected for derivation of the overall RfD as this endpoint was measured in a study that was well-conducted, utilized a test material of high purity (99.99%), and had five closely-spaced dose groups that supported characterization of the dose-response curve. In contrast, most other studies used a technical grade with ~10% or more impurities. Benchmark dose (BMD) modeling was utilized to derive the point of departure (POD) for RfD derivation (expressed as the $BMDL_{05}$). A 5% response level was chosen because of the severity of the endpoint.

A physiologically-based pharmacokinetic (PBPK) model was used to extrapolate the $BMDL_{05}$ derived from a rat study to a human equivalent dose (HED) based on RDX arterial blood concentration, which was then used for RfD derivation.

The overall RfD was calculated by dividing the $BMDL_{05\text{-}HED}$ for nervous system effects by a composite uncertainty factor (UF) of 300 to account for extrapolation from animals to humans (3), interindividual differences in human susceptibility (10), and uncertainty in the database (10).

The overall confidence in the RfD is medium based on high confidence in the principal study (Crouse et al., 2006) and medium to low confidence in the database. Confidence in the database is reduced largely because of (1) differences in test material used across studies (i.e., differences in formulation and particle size that may have affected RDX absorption and subsequent toxicity), (2) uncertainties in the influence of oral dosing methods (in particular, based on evidence that bolus dosing of RDX resulting from gavage administration induces neurotoxicity at doses lower than administration in the diet), and (3) significant limitations in the available studies to fully characterize subconvulsive neurological effects as well as developmental neurotoxicity.

Table ES-2. Summary of reference dose (RfD) derivation

Critical effect	Point of departure[a]	UF	Chronic RfD	Confidence
Nervous system effects (convulsions) 90-d F344 rat study Crouse et al. (2006)	$BMDL_{05\text{-}HED}$: 1.3 mg/kg-d	300	4×10^{-3} mg/kg-d	Medium

[a]A benchmark response (BMR) of 5% was used to derive the BMD and BMDL. The resulting POD was converted to a $BMDL_{05\text{-}HED}$ using a PBPK model based on modeled arterial blood concentration. The concentration was derived from the area under the curve (AUC) of modeled RDX concentration in arterial blood, which reflects the average blood RDX concentration for the exposure duration normalized to 24 hours.

ES.2 EVIDENCE FOR HAZARDS OTHER THAN CANCER: INHALATION EXPOSURE

No studies were identified that provided useful information on the effects observed following inhalation exposure to RDX. Of the available human epidemiological studies of RDX, none provided data that could be used for dose-response analysis of inhalation exposures. The single experimental animal study involving inhalation exposure is not publicly available, and was excluded from consideration due to significant study limitations, including small numbers of animals tested, lack of controls, and incomplete reporting of exposure levels. Therefore, the available health effects literature does not support the identification of hazards following inhalation exposure to RDX nor the derivation of an RfC.

While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support development of an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an RfC from the RfD.

ES.3 EVIDENCE FOR HUMAN CARCINOGENICITY

Under EPA's cancer guidelines (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for RDX. RDX induced benign and malignant tumors in the liver and lungs of mice (Parker et al., 2006; Lish et al., 1984) or rats (Levine et al., 1983) following long-term administration in the diet. The potential for carcinogenicity applies to all routes of human exposure.

ES.4 QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

A quantitative estimate of carcinogenic risk from oral exposure to RDX was based on the increased incidence of hepatocellular adenomas or carcinomas and alveolar/bronchiolar adenomas or carcinomas in female B6C3F_1 mice observed in the carcinogenicity bioassay in mice (Lish et al., 1984). This 2-year dietary study included four dose groups and a control group, adequate numbers of animals per dose group (85/sex/group, with interim sacrifices of 10/sex/group at 6 and 12 months), and detailed reporting of methods and results (including individual animal data). The initial high dose (175 mg/kg-day) was reduced to 100 mg/kg-day at week 11 due to high mortality.

When there is *suggestive evidence* of carcinogenicity to humans, EPA generally would not conduct a dose-response assessment and derive a cancer value. However, when the evidence includes a well-conducted study (as is the case with RDX), quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a).

An oral slope factor (OSF) was derived that considered the combination of female mouse liver and lung tumors. In modeling these data sets, the highest dose group was excluded because of the initial high mortality (loss of almost half the mice in that dose group). BMD and BMDL estimates were calculated that correspond to a 10% extra risk (ER) of either tumor. The $BMDL_{10}$ so derived was extrapolated to the HED using $BW^{3/4}$ scaling, and an OSF was derived by linear extrapolation from the $BMDL_{10\text{-HED}}$. The OSF is 0.08 per mg/kg-day, based on the liver and lung tumor response in female mice (Lish et al., 1984).

ES.5 QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

An inhalation unit risk (IUR) value was not calculated because inhalation carcinogenicity data for RDX are not available. While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an IUR from the OSF. Thus, a quantitative cancer assessment was not conducted.

ES.6 SUSCEPTIBLE POPULATIONS AND LIFESTAGES

Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Lifestage, and in particular childhood, susceptibility has not been well-studied in human or animal studies of RDX toxicity. In rats, transfer of RDX from the dam to the fetus during

gestation and to pups via maternal milk has been reported; however, reproductive and developmental toxicity studies did not identify effects in offspring at doses below those that also caused maternal toxicity. Yet, based on the primary mode-of-action for RDX exposure-induced nervous system effects (GABA receptor antagonism), and the fact that GABAergic signaling plays a prominent role in nervous system development, a significant concern is raised regarding the potential for developmental neurotoxicity. In addition, data on the incidence of convulsions and mortality provide some indication that pregnant animals may be a susceptible population, although the evidence is inconclusive. Data to suggest that males may be more susceptible than females to noncancer toxicity associated with RDX are limited. Some evidence suggests that cytochrome P450 (CYP450) enzymes may be involved in the metabolism of RDX, indicating a potential for genetic polymorphisms in these metabolic enzymes to affect susceptibility to RDX. Similarly, individuals with epilepsy or other seizure syndromes that have their basis in genetic mutation to $GABA_A$ receptors may represent another group that may be susceptible to RDX exposure; however, there is no information to indicate how genetic polymorphisms may affect susceptibility to RDX.

LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

SR.1 LITERATURE SEARCH AND SCREENING STRATEGY

A literature search and screening strategy was applied to identify literature related to characterizing the health effects of hexadydro-1,3,5-trinitro-1,3,5-triazine (RDX). This strategy consisted of a search of online scientific databases and other sources, casting a wide net in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the chronic health effects of RDX, and the remaining references were sorted into categories for further evaluation.

The literature search for RDX was conducted in four online scientific databases—PubMed, Toxline, Toxcenter, and Toxic Substances Control Act Test Submissions (TSCATS). The initial search was performed in April 2012, and literature search updates were conducted in February 2013, January 2014, January 2015, and May 2016. Searches of TSCATS were performed in February 2013, January 2015, and May 2016 only. In addition, a post-peer review literature search was conducted in November 2017 (described below). The detailed pre-peer review search approach for these databases, including the query strings, and the numbers of citations identified per database are provided in Appendix B, Table B-1. The Department of Defense has conducted several unpublished toxicological studies on RDX; to ensure that all such studies were located, the Defense Technical Information Center (DTIC) database, a central online repository of defense-related scientific and technical information within the Department of Defense, was also searched. A separate strategy was applied in searching DTIC because of limitations in the classification and distribution of materials in DTIC; the detailed search strategy is described in Appendix B, Table B-2. Searches of the five online databases identified 1,247 citations (after electronically eliminating duplicates). The computerized database searches were supplemented by reviewing online regulatory sources, performing "forward" and "backward" searches of Web of Science (see Appendix B, Table B-3), and adding additional references that were identified during the development of the Toxicological Review (including submissions from the Department of Defense); 34 citations were obtained using these additional search strategies. In total, 1,281 citations were identified using online scientific databases and additional search strategies.

The U.S. Environmental Protection Agency (EPA) requested public submissions of additional information in 2010 (75 FR 76982; December 10, 2010). No submissions were received in response to these calls for data. EPA also issued a request to the public for additional information in a Federal Register Notice in 2013 (78 FR 48674; August 9, 2013), and established a

docket for public comment (EPA-HQ-ORD-2013-0430; available at www.regulations.gov) maintained through the development of the assessment.

The citations identified using the search strategy described above were screened based on title and abstract, and when needed, full text for pertinence to examining the health effects of chronic RDX exposure. The process for screening the literature is described below and is shown graphically in Figure LS-1 and on the RDX project page on EPA's Health and Environmental Research Online (HERO) website at: https://hero.epa.gov/index.cfm/project/page/project_id/2216.[7] The objective of this manual screen was to identify sources of primary human health effects data (i.e., human data and pertinent data from in vivo animal models) and other sources of primary data that inform the assessment of RDX health effects (i.e., genotoxicity and other mechanistic studies and toxicokinetic studies). These data sources are represented by the bottom three boxes in Figure LS-1. Inclusion and exclusion criteria used to manually screen the references in order to identify health effect studies (i.e., the green boxes with dashed boarders in Figure LS-1) are provided in Table LS-1.

All studies that provided data on adsorption, distribution, metabolism, or elimination, physiologically-based pharmacokinetic (PBPK) models, or relevant RDX mode of action (MOA) were tracked and considered in the assessment.

Reviews and other sources of RDX information (e.g., studies with exposure level information) that did not meet the inclusion criteria for primary health effect studies in Table LS-1 were tracked as "Secondary Literature and Sources of Other RDX Information," and were considered as appropriate during development of this assessment. Studies identified as "Excluded/Not on Topic" (see exclusion criteria in Table LS-1) were not further considered in this assessment.

The results of this literature screening are described below and graphically in Figure LS-1:

- 21 references (including both human and animal studies) were identified as sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays.

[7]HERO is a database of scientific studies and other references used to develop EPA's assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than two million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO.

Studies were assigned (or "tagged") to a given category in HERO that best reflected the primary content of the study. In general, studies were not assigned multiple tags in order to simplify the tracking of references. Nevertheless, the inclusion of a citation in a given category (or tag) did not preclude its use in one or more other categories. For example, Woody et al. (1986), a case report of accidental ingestion of RDX by a child, was tagged to the human case reports under Supplementary Studies in Figure LS-1. This case report also provides pharmacokinetic data and was a pertinent source of information on RDX toxicokinetics, but was not assigned a second tag for toxicokinetics.

- 25 references were identified as sources of supplementary health effects data, including experimental animal studies involving acute or short-term exposures or dermal exposure, and human case reports. Studies investigating the effects of acute/short-term and dermal exposures and case reports are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposure. Therefore, information from these studies was not extracted into evidence tables. Nevertheless, these studies were still considered as possible sources of supplementary health effects information.

- 91 references provided information on nonmammalian species (tagged as ecosystem studies) that can inform the hazard evaluation or potential MOA, and specifically in the case of RDX, the conservation of neurotoxic response across phylogenetically diverse organisms. Information from these studies was not extracted into evidence tables; however, these studies were tracked as supplementary health effects information.

- 47 references were identified as sources of mechanistic and toxicokinetic data; these included 19 studies describing PBPK models and other toxicokinetic information, 11 studies providing genotoxicity information, and 17 studies pertaining to other mechanistic information. Information from these studies was not extracted into evidence tables; however, these studies supplemented the assessment of RDX health effects. Specifically, mechanistic studies were used in the evaluation of potential MOAs and to develop the mechanistic evidence stream that was considered in the overall integration of evidence for assessing hazard. Toxicokinetic data were used to inform extrapolation of experimental animal findings to humans.

- 190 references were identified as secondary literature (e.g., reviews and other agency assessments), peer review reports of primary (unpublished) health effect studies, or contextual information (e.g., studies with RDX exposure information). These references were kept as additional resources for development of the Toxicological Review.

- 907 references were identified as not being pertinent (or not on topic) to an evaluation of the chronic health effects of RDX and were excluded from further consideration (see Figure LS-1 and Table LS-1 for exclusion criteria).

SR.1.1. Post-peer Review Literature Search Update

A post-peer review literature search update was conducted in PubMed, Toxline, TSCATS, and DTIC for the period May 2016 to November 2017 using a search strategy consistent with previous literature searches (see Appendix B, Tables B-1 and B-2). Toxcenter, used in previous searches, was not searched in the November update. Toxcenter is a proprietary, fee-based database produced by Chemical Abstract Service. Evaluation of the references retrieved using Toxcenter in searches conducted through May 2016 revealed that this database did not locate pertinent references not already identified by other online databases. Results of the November 2017 literature search update are summarized in Appendix B, Tables B-1 and B-2.

Consistent with the IRIS Stopping Rules (https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf), manual screening of the literature search update focused on identifying new studies that might

change a major conclusion of the assessment. No potentially pertinent references were identified in the post-peer review literature search.

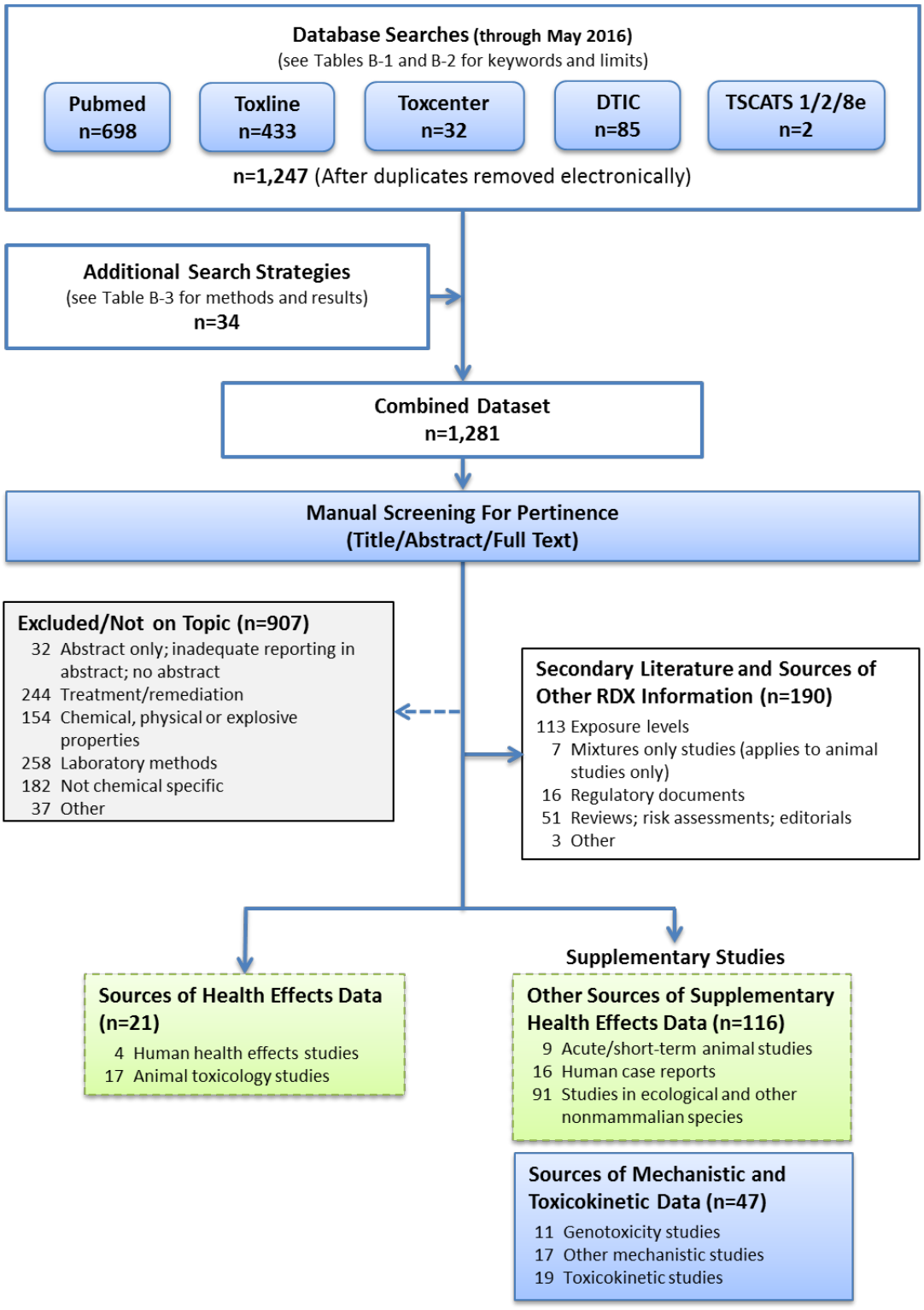


Figure LS-1. Summary of literature search and screening process for RDX.

The numbers on this figure match the HERO project page as of 5/1/2018. See text for search strategy and results of an updated literature search conducted in November 2017 (post peer review).

Table LS-1. Inclusion-exclusion criteria for health effect studies[a]

	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, monkey, dog • In vitro studies -- tracked as supplementary information • Ecological and nonmammalian species – tracked as supplementary information	
Exposure	• Exposure is to RDX • Exposure is measured in an environmental medium (e.g., air, water, diet) • Exposure via oral or inhalation routes	• Study population is not exposed to RDX • Exposure to a mixture only (applied to animal studies only) • Exposure via injection (e.g., intravenous [i.v.])[b]
Outcome	• Study includes a measure of one or more health effect endpoints, including effects on the nervous, urinary, musculoskeletal, cardiovascular, immune, and gastrointestinal systems, reproduction, development, liver, eyes, and cancer • Mechanistic and toxicokinetic studies -- tracked as supplementary information	
Other		• Reviews, regulatory documents (i.e., not primary sources of health effect data)[b] • Exposure levels[b] • Not on topic, including: o Abstract only, inadequately reported abstract, or no abstract, and not considered further because study was not potentially relevant o Bioremediation, biodegradation, or chemical or physical treatment of RDX and other munitions, including evaluation of wastewater treatment technologies and methods for remediation of contaminated water and soil o Chemical, physical, or explosive properties, including studies of RDX crystal quality, energetics characteristics, sublimation kinetics, isotope ratios, and thermal decomposition and other explosive properties o Analytical methods for measuring/detecting/ remotely sensing RDX in environmental media, and use in sample preparations and assays o Not chemical specific (studies that do not involve testing of RDX)

	Inclusion criteria	Exclusion criteria
		o Other studies not informative for evaluating RDX health effects and not captured by other exclusion criteria, including: -- Superfund site records of decision that describe remedial action plans for waste sites -- characterization of waste sites contaminated by explosives -- foreign language studies where translation was not warranted because, based on title or abstract, the added value to the evaluation of RDX health effects was considered small (e.g., Chinese paper of case reports of RDX poisonings) -- duplicate studies not previously identified during electronic screening

[a]Inclusion/exclusion criteria were designed to identify sources of primary human health effects data (i.e., human data and pertinent data from in vivo animal models).
[b]Studies that met this exclusion criterion were not considered a primary source of health effects or supplementary health effects data; however, these studies were tracked and considered as other sources of information potentially useful in assessing the health effects of RDX, including potential MOAs.

The documentation and results for the literature search and screen, including the specific references identified using each search strategy and tags assigned to each reference based on the manual screen, can be found on the HERO website on the RDX project page at: (https://hero.epa.gov/index.cfm/project/page/project_id/2216).

SR.2 STUDY EVALUATION AND SELECTION OF CRITICAL STUDIES

SR.2.1. Selection of Critical Studies

In order to systematically summarize the important information from the primary health effects studies in the RDX database, evidence tables were constructed in a standardized tabular format as recommended by the NRC (2011). Of the studies that were retained after the literature search and screen, 21 were categorized as "Sources of Health Effects Data" (see Figure LS-1, Table LS-1) and were considered for extraction into evidence tables for hazard identification in Chapter 1.

A study was not subject to a more thorough review of study quality and was not presented in evidence tables if flaws in its design, conduct, or reporting were so great that the results would not be considered credible (e.g., studies where concurrent control information is lacking). Such study design flaws are discussed in a number of EPA's guidelines (see https://www.epa.gov/iris/backgrd.html and Section 4 of the Preamble). For RDX, four studies were considered uninformative and were removed from further consideration in the assessment because of fundamental issues with study design, conduct, or reporting. The specific studies and basis for considering the studies to be uninformative are summarized in Table LS-2.

Table LS-2. Studies determined not to be informative because of significant issues with design, conduct, or reporting

Reference	Rationale for exclusion
Haskell Laboratories (1942); repeat dose studies in dogs and rats	Inadequate reporting of study design (e.g., limited exposure information, breed of dog was not reported) and results; sections of document were illegible. Deficiencies in experimental design of dog study (e.g., investigation of only blood pressure in 3 dogs exposed for 2, 14, or 16 weeks; no separate control). Rat study included only 10 rats treated 41 times with RDX over an unspecified exposure duration; only body weight and survival findings were reported.
von Oettingen et al. (1949); 10-wk oral study in rats	No control group; strain of rat was not reported. Note: other studies included in the paper by von Oettingen et al. (1949) were retained; results of these studies are included in evidence tables.
ATSDR (1996); Disease prevalence study in residential population	Study of a population residing in two neighborhoods where RDX had been detected in well water. The study was conducted 7 yrs after residents were provided the opportunity to connect to a municipal water supply. Only one target-area household reported using private well water for bathing and cooking at the time of the health study. The study was not considered informative because the design was not able to adequately define the exposed population.
Unpublished report (dated 1944) from the DTIC database; Human and animal data	One section of the report describes a human case series with no referent group. Issues with the inhalation experimental animal studies included lack of control groups, incomplete information on exposure levels, and inadequate reporting of results. [Because this report is classified as a limited distribution document in the DTIC database, it was not added to the HERO project page for RDX.]

The health effects literature for RDX is not extensive. With the exception of the studies listed in Table LS-2 (i.e., those determined to be uninformative), all human and experimental animal studies of RDX involving repeated exposure were considered in assessing the evidence for health effects associated with chronic exposure to RDX.

Studies that contain pertinent information for the toxicological review and augment hazard identification conclusions, such as genotoxicity and other mechanistic studies, studies describing the toxicokinetics of RDX, human case reports, and experimental animal studies involving exposures of acute/short-term duration or routes of exposure other than oral and inhalation, were not included in evidence tables. Nevertheless, these studies were considered, where relevant, in the evaluation of RDX health hazards.

SR.2.2. Study Evaluation

For this assessment, primary sources of health effects data consisted of three human studies[8] and 16 reports[9] presenting results of experimental animal studies. These studies were evaluated using the study quality considerations described below that addressed aspects of design, conduct, or reporting that could affect the interpretation of results, overall contribution to the synthesis of evidence, and determination of hazard potential as noted in various EPA guidance documents (U.S. EPA, 2005a, 2002, 1994). The objective was to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

Additionally, a number of general questions, presented in Table LS-3, were considered in evaluating the animal studies. Much of the key information for conducting this evaluation can be determined based on study methods and how the study results were reported. Importantly, the evaluation at this stage does not consider the direction or magnitude of any reported effects.

[8]Two reports with human data were determined not to be informative; see Table LS-2. The study by ATSDR (1996) was included in HERO and in Figure LS-1. The unpublished report from the DTIC database was not included in either HERO or Figure -1 because this report is classified as a limited distribution document in DTIC. This accounts for the three human studies being reviewed for study evaluation rather than the four identified in the literature search (see Figure LS-1).

[9]One of 17 animal toxicity studies identified in Figure 1 (Haskell Laboratories (1942) was determined to be uninformative; see Table LS-2. This study was included in HERO and Figure LS-1, but was not considered a primary source of health effects data and was not carried forward for further review.

Also, it should be noted that the number of reports of experimental animal studies does not equal the number of studies for several reasons. The results of some studies were documented in multiple reports (e.g., a 2-year study in F344 rats by Levine et al. (1983) was published in three volumes). The Cholakis et al. (1980) study included, in a single report, subchronic studies in rats and mice, a 2-generation reproductive toxicity study in rats, and developmental toxicity studies in rats and rabbits. A 13-week toxicity study of RDX in rats was reported initially as a laboratory report study (Levine et al., 1981a), and results were subsequently included in two published papers. A Pathology Working Group review of the female mouse liver tumor data in the Lish et al. (1984) 2-year bioassay was provided as a study report and subsequently as a published paper.

Table LS-3. Considerations and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Considerations (relevant information extracted into evidence tables)
Test animal	Suitability of the species, strain, sex, and source of the test animals
Experimental design	Suitability of animal age/lifestage at exposure and endpoint testing; periodicity and duration of exposure (e.g., hrs/d, d/wk); timing of endpoint evaluations; and sample size and experimental unit (e.g., animals, dams, litters)
Exposure	Characterization of test article source, composition, purity, and stability; suitability of the control (e.g., vehicle control); documentation of exposure techniques (e.g., route, chamber type, gavage volume); verification of exposure levels (e.g., consideration of homogeneity, stability, analytical methods)
Endpoint evaluation	Suitability of specific methods for assessing the endpoint(s) of interest
Results presentation	Data presentation for endpoint(s) of interest (including measures of variability) and for other relevant endpoints needed for results interpretation (e.g., maternal toxicity, decrements in body weight in relation to organ weight)

Information on study features related to this evaluation is reported in evidence tables and was considered in the synthesis of evidence. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant. If EPA's interpretation of a study differed from that of the study authors, the assessment discusses the basis for the difference.

The general findings of this evaluation are presented in the remainder of this section and discussed in the relevant health effect sections in Section 1.2.

Human Studies

The body of literature on RDX includes three studies of populations occupationally exposed to RDX (one case-control and two cross-sectional studies) (West and Stafford, 1997; Ma and Li, 1993; Hathaway and Buck, 1977). To varying degrees, these epidemiology studies are limited in their ability to assess the relationship between RDX exposure and the incidence of human health effects. Some studies lacked information related to study design, such as a clear definition of the study population, while others did not include a comprehensive exposure assessment or details regarding potential confounders. All three studies had small sample sizes (60–69 exposed workers in the cross-sectional studies and 32 cases in the case-control study), which limits their statistical power when comparing exposed workers or cases and unexposed or control participants.

The study by Ma and Li (1993) of Chinese industrial workers provided limited information on participant recruitment, selection, and participation rate; the available information was not adequate to evaluate the potential for selection bias. Also, no information on adjustment for co-exposure to trinitrotoluene (TNT) or other neurological risk factors (e.g., alcohol consumption) was provided. The study by Hathaway and Buck (1977) included details on exposure assessment, but did not provide information on length of employment or other metrics that could be used to

ascertain duration of exposure. In the case-control study by West and Stafford (1997), RDX was identified as one of the many chemicals that workers may have been exposed to in the ordnance factory. Thus, there is a potential for co-exposure to other chemicals that may elicit the observed effects. The methodological limitations in these three studies were considered in the synthesis of evidence for each of the health effects and in reaching determinations of hazard (see Section 1.2).

In addition to the three epidemiological studies, the human health effects literature includes 16 case reports that describe effects following acute exposure to RDX. Case reports can suggest organ systems and health outcomes that might be related to RDX exposure but are often anecdotal, and typically describe unusual or extreme exposure situations; thus, they provide little information that would be useful for characterizing chronic health effects or deriving toxicity values. Therefore, RDX case reports were only briefly reviewed; a critical evaluation was not undertaken. A summary of these case reports is provided in Appendix C, Section C.2.

Experimental Animal Studies

The oral toxicity database for RDX includes three chronic studies in rats and mice, eight subchronic studies in rats, mice, dogs, and monkeys, two shorter-term studies in dogs and rats, one two-generation reproductive toxicity study in the rat, four developmental toxicity studies in rats and rabbits, and a single-exposure study of audiogenic seizures in rats (Table LS-4).

Table LR-4. Summary of experimental animal database

Study category	Study duration, species/strain, and oral administration method
Chronic	2-Yr study in B6C3F$_1$ mice (diet) (Lish et al., 1984) 2-Yr study in Sprague-Dawley rats (diet) (Hart, 1976) 2-Yr study in F344 rats (diet) (Levine et al., 1983)
Subchronic	13-Wk study in B6C3F$_1$ mice, experiment 1 (diet) (Cholakis et al., 1980) 13-Wk study in B6C3F$_1$ mice, experiment 2 (diet) (Cholakis et al., 1980) 13-Wk study in F344 rats (diet) (Cholakis et al., 1980) 13-Wk study in F344 rats (diet) (Levine et al., 1990; Levine et al., 1981a, b) 13-Wk study in F344 rats (gavage) (Crouse et al., 2006) 13-Wk study in rats, strain not specified (diet) (von Oettingen et al., 1949) 13-Wk study in beagle dogs (diet) (Hart, 1974) 13-Wk study in monkeys (gavage) (Martin and Hart, 1974) 6-Wk study in dogs, breed not specified (diet) (von Oettingen et al., 1949) 30-D study in Sprague-Dawley rats (gavage) (MacPhail et al., 1985)
Reproductive	2-Generation reproductive toxicity study in CD rats (diet) (Cholakis et al., 1980)
Developmental	Developmental study (gestational days [GDs] 6–19) in F344 rats (gavage) (Cholakis et al., 1980) Developmental study (GDs 6–15) in Sprague-Dawley rats, range-finding (gavage) (Angerhofer et al., 1986) Developmental study (GDs 6–15) in Sprague-Dawley rats (gavage) (Angerhofer et al., 1986) Developmental study (GDs 7–29) in New Zealand White (NZW) rabbits (gavage) (Cholakis et al., 1980)
Nervous system	8-Hr study of audiogenic seizures in Long Evans rats (gavage) (Burdette et al., 1988)[a] Acute EEG and in vitro studies of RDX evoked seizure activity in Sprague-Dawley male rats (Williams et al., 2011)

[a]As an 8-hour study, Burdette et al. (1988) was tagged in Figure LS-1 and the HERO database as "Other Sources of Supplementary Health Effects Data," but was nevertheless included in the evidence table for nervous system effects of RDX as the only study to examine potential effects of RDX on seizure threshold.

With the exception of two studies (Levine et al., 1990; von Oettingen et al., 1949), these toxicity studies are available only as unpublished contract laboratory reports. Peer reviews of four unpublished studies identified as most informative to the assessment of the health effects of RDX—the 2-year bioassays by Levine et al. (1983) and Lish et al. (1984), the subchronic toxicity study by Crouse et al. (2006), and the collection of repeat-dose studies reported in (Cholakis et al., 1980)—were conducted by Versar, Inc. or Eastern Research Group, Inc. for EPA. The reports of the peer reviews (U.S. EPA, 2017, 2012c) are available at https://epa.gov/hero. The peer reviewers generally concluded that the 2-year bioassay reports provided useful information on the toxicity of RDX, noting that there were limitations in interpretation due to aspects of the histopathological analysis and the statistical approaches employed. The peer reviewers similarly determined that the report by Crouse et al. (2006) provided useful information on RDX toxicity, including an array of endpoints for neurotoxicity and immunotoxicity, although the assessment of neurotoxicity in the study could have been improved with more histological evaluation as well as additional behavioral assessment. (U.S. EPA, 2012c). The peer review report of the repeat-dose studies in Cholakis et al.

(1980) found that the studies were generally appropriate and adequate for evaluating the toxicity of RDX, and conducted consistent with standards in place at the time the experiments were conducted (U.S. EPA, 2017).

Only one unpublished inhalation study of RDX (dated 1944) was identified. This inhalation study was considered uninformative and was excluded from consideration in the development of the Toxicological Review because of study design issues (including lack of a control group, incomplete information on exposure levels, and inadequate reporting, see Appendix B and Table LS-2). Therefore, evaluation of the experimental animal database for RDX is limited to studies of oral toxicity. An evaluation of the oral toxicity literature, organized by general methodological features, is provided in the remainder of this section.

Test animal

The RDX database consists of health effect studies conducted in multiple strains of rats (F344, Sprague-Dawley, CD), mice ($B6C3F_1$), dogs (beagle), and monkeys. The species and strains of animals used are consistent with those typically used in laboratory studies. All of these species or strains were considered relevant to assessing the potential human health effects of RDX. The species, strain, and sex of the animals used are recorded in the evidence tables.

Other studies of RDX were identified that used nonstandard species, including deer mice (*Peromyscus maniculatus*), western fence lizards (*Sceloporus occidentalis*), prairie voles (*Microtus ochrogaster*), and northern bobwhite quail (*Colinus virginianus*). These studies provide information relevant to RDX toxicokinetics and mechanism of action on the nervous system, but not health effects data. Therefore, these studies (tagged under Supplementary Studies; see Figure LS-1) are not included in evidence tables, but are discussed where relevant in the assessment.

Experimental design

General aspects of experimental design were evaluated for all studies that included health effects data to determine if they were appropriate for evaluation of specific endpoints. Key features of the experimental design, including the periodicity and duration of exposure, timing of exposure (e.g., gestational days for developmental studies), experimental group sample sizes, and interim sacrifices are summarized in the evidence tables. Note that sample size was not a basis for excluding a study from consideration, as studies with a small number of animals can still inform the consistency of effects observed for a specific endpoint. Nevertheless, the informativeness of studies with small sample sizes, e.g., three animals/sex/group in the case of Hart (1974) and Martin and Hart (1974), was reduced. Elements of the experimental setup that could influence interpretation of study findings are discussed in the relevant hazard identification sections of the assessment.

Exposure

Studies were evaluated with respect to the reliability of the reported exposure to RDX, focusing on considerations related to properties of the test material and confirmation of the administered dose.

Two properties of the RDX test materials that varied across experimental animal studies and that were taken into consideration in evaluating the evidence for RDX hazards are the particle size and purity of the test material. The purity of RDX used in health effects studies varied from 84 to 99.99%. The major contaminant was octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), which is produced during manufacturing. The majority of studies used RDX with ~10% impurities; only Crouse et al. (2006) used 99.99% pure RDX as a test material in their study. The toxicity of HMX was assessed by the Integrated Risk Information System (IRIS) Program in 1988 (https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=311); histopathological changes in the liver in male F344 rats and in the kidney in female rats were reported in a 13-week feeding study. No chronic studies were available to evaluate the carcinogenicity of HMX. The presence of the impurities introduces some uncertainty in attribution of toxicity to RDX. However, consistency in the doses at which some toxic effects were seen across studies suggests that the uncertainty associated with the use of less pure test materials may be relatively small. Evidence of neurotoxic effects in the study with 99.99% pure RDX occurred at doses of 8–15 mg/kg-day; studies with less pure RDX reported similar symptoms at doses ≥20 mg/kg-day. It should be noted that the test materials employed in these studies (i.e., with ~10% impurities) are consistent with the purity of RDX that would be released into the environment.

Differences in milling procedures used to generate the test material resulted in the use of RDX of varying particle sizes across studies. Some studies utilized a test material with a relatively fine particle size (majority of particles <66 µm in size), while others used a test material with comparatively coarse particle size (~200 µm particle size). Differences in particle size across studies could result in different rates of absorption of RDX into the blood stream, which could account for differences in response observed across studies, including neurotoxicity.

Information on test material purity and particle size, as provided by study authors, is reported in the evidence tables, and was considered in evaluating the toxicity of RDX. The lack of characterization of the test material in the studies by Hart (1974), Hart (1976), and Martin and Hart (1974) was considered a deficiency.

Only four studies assayed dose preparations to determine how close the actual RDX concentrations were to target (nominal) concentrations (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Cholakis et al., 1980). Cholakis et al. (1980) described the largest difference between target and actual dose concentrations; assays of the suspensions prepared for the oral (gavage) developmental toxicity study showed RDX dosing suspensions ranging from 36 to 501% of the target concentrations (Appendix I of Cholakis et al. (1980)). Assays of RDX-treated feed used in the 90-day studies in rats and mice and the two-generation reproductive toxicity study in rats showed RDX concentrations that were 78 to 209% of target concentrations (Appendix I of Cholakis et al. (1980)). The authors stated, "maintaining uniform suspensions was not always easy." In the 90-day oral (gavage) toxicity study in rats (Crouse et al., 2006), fresh dose suspensions were prepared monthly, mixed with a magnetic stir bar until a uniform suspension was obtained, and

remixed each day during the dosing procedure; each dose suspension was analyzed prior to use. RDX concentrations varied from 83 to 114%; the 114% suspension was adjusted to 100% before administration (Crouse et al., 2006). In 30 assays performed over the course of a 24-month bioassay in mice, Lish et al. (1984) determined dietary concentrations of RDX to be 73 to 103% of target concentrations. In 32 assays performed over a 24-month bioassay in rats, Levine et al. (1983) reported that dietary concentrations of RDX were 67% to 122% of target concentrations. In the remaining studies, failure to analyze or report actual concentrations of RDX in the dosing suspension or test diet is considered a deficiency.

Endpoint evaluation procedures

Some methodological considerations used to evaluate studies of RDX toxicity are outcome specific—in particular, effects on the nervous system and development. Outcome-specific methodological considerations are discussed in the relevant health effect sections in Section 1.2. For example, many of the studies that noted neurotoxicity in the form of seizures or convulsions were not designed to assess that specific endpoint and reported the number of animals with seizures as part of clinical observations that, in general, were recorded only once daily. This frequency of observations could have missed neurobehavioral events or identify subtler subconvulsive behaviors. While these studies can provide qualitative evidence of neurotoxicity, they may have underestimated the true incidence of seizures or convulsive behaviors because they were not designed to systematically evaluate neurotoxic outcomes.

Results presentation

In evaluating studies, consideration was given to whether data were reported for all endpoints specified in the methods section and for all study groups, and whether any data were excluded from presentation or analysis. For example, it was noted where histopathological analysis was limited to control and high-dose groups, a study reporting feature that limited the ability to identify dose-related trends. In limited cases, EPA performed additional statistical analysis to identify trends or refine analyses consistent with EPA guidance (e.g., analyzing developmental data sets on a per litter basis rather than by individual fetus). Study results have been extracted and presented in evidence tables.

Notable features of the RDX database

Three 2-year toxicity bioassays of RDX are available as unpublished laboratory studies (Lish et al., 1984; Levine et al., 1983; Hart, 1976). The bioassays by Levine et al. (1983) in the rat and by Lish et al. (1984) in the mouse were conducted in accordance with Food and Drug Administration (FDA) Good Laboratory Practices (GLPs) in place at the time of the studies. Both studies included interim sacrifices (at 6 and 12 months). Complete histopathological examinations were performed on all animals in the control and high-dose groups; however, only a subset of tissues was examined in the mid-dose groups (including brain, gonads, heart, liver, kidneys, spleen, and spinal cord in both species, and lungs and tissue masses in the mouse), limiting the ability to identify dose-related

trends for tissues with incomplete histopathology. Additionally, in the mouse bioassay by Lish et al. (1984), the initial high dose (175 mg/kg-day) was reduced to 100 mg/kg-day at week 11 because of high mortality, thereby reducing the number of high-dose animals on study for the full 2 years of dosing (see Table LS-5).

An earlier unpublished 2-year study in rats by Hart (1976) used a dose range that was lower than the Levine et al. (1983) and Lish et al. (1984) bioassays. Histopathology findings were limited by the lack of pathology examinations in the mid-dose groups and lack of individual time of death, which impacts the ability to interpret the histopathology data. In addition, a heating system malfunction on days 75−76 of the study resulted in the death of 59 rats from the control and treatment groups, thereby reducing the number of animals in the study (see Table LS-5).

Experimental animal toxicity studies of RDX involving less-than-lifetime exposure (Crouse et al., 2006; Angerhofer et al., 1986; MacPhail et al., 1985; Levine et al., 1981a; Cholakis et al., 1980; Hart, 1974; Martin and Hart, 1974; von Oettingen et al., 1949) were published or reported between the years 1949 and 2006, and differences in robustness of study design, conduct, and reporting reflect that time span. All but two of the eight short-term and subchronic toxicity studies of RDX are available as unpublished laboratory studies; published studies include von Oettingen et al. (1949) and Levine et al. (1981a), a laboratory report of a 13-week study of RDX in F344 rats with subsets of the data subsequently published as Levine et al. (1981b) and Levine et al. (1990). The majority of studies conducted histopathological examinations on only some of the experimental groups (e.g., control and high dose).

Some of the more important limitations in study design, conduct, and reporting of experimental animal toxicity studies of RDX are summarized in Table LS-5. Limitations of these studies as well as the study evaluation consideration described in this section were taken into consideration in evaluating and synthesizing the evidence for each of the health effects in Section 1.2.

Table LS-5. Experimental animal studies considered less informative because of certain study design, conduct, or reporting limitations

References	Study design, conduct, and reporting limitations
Lish et al. (1984) 2-yr mouse study	The initial high dose (175 mg/kg-d) was reduced to 100 mg/kg-d at wk 11 due to high mortality. Mortality of surviving mice was similar to controls after dose reduction.
Hart (1976) 2-yr rat study	A heating system malfunction on d 75−76 of the study resulted in the deaths of 59 rats from the control and treatment groups. Dead animals were subsequently eliminated from the analysis. Interpretation of the histopathology findings was limited by the lack of pathology examinations in the mid-dose groups and lack of individual time of death. Test material poorly characterized; purity was not reported.
Cholakis et al. (1980) 13-wk mouse study (Experiment 1)	The dose range was too low to produce effects in mice. Assays of RDX-treated feed showed RDX concentrations between 123% and 209% of target concentrations. Histopathological examinations were not performed.
Cholakis et al. (1980) 13-wk mouse study (Experiment 2)	Nonstandard dosing regimen followed: 0, 40, 60, or 80 mg/kg-d for 2 wks. For the next 11 wks, the dosing was inverted, so that the 40 mg/kg-d group received 320 mg/kg-d, the 60 mg/kg-d group received 160 mg/kg-d, and the 80 mg/kg-d group continued to receive the same dose. The rationale for this dosing regimen was not provided in the study report.
Cholakis et al. (1980) Developmental study in rats	Large differences were reported between target and actual dose concentrations in the suspensions prepared for oral (gavage) administration; actual RDX concentrations in dosing suspensions ranged from 36 to 501% of the target concentrations.
Levine et al. (1981a) 13-wk rat study	Analysis of one lot of rodent feed showed measurable levels of contaminants, including chlorinated pesticides (dieldrin, heptachlor epoxide, beta-hexachlorocyclohexane [BHC], and dichlorodiphenyltrichloroethane [DDT]), polychlorinated biphenyls (PCBs), and organophosphates (methyl parathion, carbophenothion, and disulfeton).
Martin and Hart (1974) 13-wk monkey study	The species of monkey is unclear (either Cynomolgus or Rhesus). Some test subjects may have had variable dosing due to emesis. Small sample size per dose group (n = 3). Test material poorly characterized; purity was not reported.
von Oettingen et al. (1949) 12-wk rat study	The strain of rat was not reported. Only gross observations were made at autopsy.
von Oettingen et al. (1949) 6-wk dog study	The breed of dog was not reported. Only gross observations were made at autopsy.

1

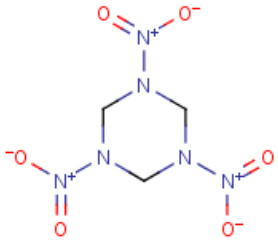
1. HAZARD IDENTIFICATION

1.1. OVERVIEW OF CHEMICAL PROPERTIES AND TOXICOKINETICS

1.1.1. Chemical Properties

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a member of the nitramine class of organic nitrate explosives (Boileau et al., 2003; Bingham et al., 2001) and is not found naturally in the environment. RDX exists as a white, crystalline solid Bingham et al. (2001). It has low solubility in water (Yalkowsky and He, 2003) and slowly volatilizes from water or moist soil (ATSDR, 2012). The normalized soil organic carbon/water partition coefficient (K_{OC}) values for RDX indicate a potential for RDX to be mobile in soil (Spanggord et al., 1980). The vapor pressure suggests that RDX will exist as particulate matter in air and be removed by both wet and dry deposition (Spanggord et al., 1980). Information on physiochemical properties for RDX is available at EPA's Chemistry Dashboard (https://comptox.epa.gov/dashboard/) and is summarized in Table 1-1.

Table 1-1. Chemical identity and physicochemical properties of RDX from EPA's Chemistry Dashboard

Characteristic or property	Value	
Chemical structure		
CASRN	121-82-4	
Synonyms	1,3,5-triaza-1,3,5-trinitrocyclohexane; 1,3,5-triazine, hexahydro-1,3,5-trinitro-; 1,3,5-trinitro-1,3,5-triazacyclohexane; 1,3,5-trinitro-1,3,5-triazinane; 1,3,5-trinitrohexahydro-1,3,5-triazine; 1,3,5-trinitrohexahydro-s-triazine; 1,3,5-trinitroperhydro-1,3,5-triazine; cyclonite; cyclotrimethylenenitramine; cyclotrimethylenetrinitramine; hexahydro-1,3,5-trinitro-1,3,5-s-triazine; hexahydro-1,3,5-trinitro-1,3,5-triazine; hexahydro-1,3,5-trinitro-s-triazine; hexogen; perhydro-1,3,5-trinitro-1,3,5-triazine; RDX; Research Development Explosive; Royal Demolition eXplosive; sym-trimethylene trinitramine; s-triazine, hexahydro-1,3,5-trinitro-; trimethylenetrinitramine; trinitrocyclotrimethylene triamine; trinitrotrimethylenetriamine (see https://comptox.epa.gov/dashboard for additional synonyms)	
Molecular formula	$C_3H_6N_6O_6$	
Molecular weight	222.117	
	Average experimental value[a]	**Average predicated value[a]**
Flash point (°C)	--	388
Boiling point (°C)	--	407
Melting point (°C)	205	162
Log K_{OW}	0.87	-0.425
Water solubility (mol/L)	2.69×10^{-4}	8.37×10^{-3}
Density (g/cm^3)	--	1.84
Henry's law constant (atm-m^3/mole)	--	2.53×10^{-6}
Vapor pressure (mm Hg at 20˚C)	4.10×10^{-9}	3.76×10^{-9}

[a]Median values and ranges for physical chemical properties of RDX are also provided on the Chemistry Dashboard at https://comptox.epa.gov/dashboard/.

RDX degrades in the environment and can be subject to both photolysis (Sikka et al., 1980; Spanggord et al., 1980) and biodegradation (Funk et al., 1993; McCormick et al., 1981). RDX is metabolized by microbial nitroreductases to form the N-nitroso derivatives hexahydro-1-nitroso-

3,5-dinitro-1,3,5- triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) (Jaligama et al., 2013; Halasz et al., 2012; Smith et al., 2006; Meyer et al., 2005; Beller and Tiemeier, 2002). 4-Nitro-2,4-diazabutanal (NDAB) and methylenedinitramine (MEDINA) have also been detected as microbial metabolites of RDX (Halasz et al., 2012; Fuller et al., 2010).

1.1.2. Toxicokinetics

RDX is absorbed following exposure by oral and inhalation routes (see Appendix C, Section C.1.1). Studies in experimental animals indicate that oral absorption rates can range from approximately 50 to 90% (Krishnan et al., 2009; Guo et al., 1985; Schneider et al., 1978, 1977), with the rate and extent of absorption dependent on the physical form of RDX (i.e., the increased surface area associated with finely powdered RDX allows for increased absorption) and the dosing preparation or matrix (Bannon et al., 2009a; Krishnan et al., 2009; Crouse et al., 2008; Bannon, 2006; Guo et al., 1985; MacPhail et al., 1985; Schneider et al., 1977). Dermal absorption of RDX has been demonstrated in in vitro studies using human and pig skin (Reddy et al., 2008; Reifenrath et al., 2008).

RDX is systemically distributed, including to the brain (i.e., RDX can cross the blood:brain barrier), heart, kidney, liver, and fat (Musick et al., 2010; Bannon et al., 2006; MacPhail et al., 1985; Schneider et al., 1977). In rats, RDX can be transferred from dam to fetus across the placental:blood barrier, and has been identified in maternal milk (Hess-Ruth et al., 2007).

The metabolism of RDX in humans has not been investigated. Studies in experimental animals indicate that metabolism of RDX is extensive and includes denitration, ring cleavage, and generation of CO_2 possibly through cytochrome P450 (CYP450) (Musick et al., 2010; Major et al., 2007; Fellows et al., 2006; Bhushan et al., 2003; Schneider et al., 1978, 1977).

RDX and metabolites are eliminated primarily via urinary excretion and exhalation of CO_2 (Sweeney et al., 2012a; Musick et al., 2010; Krishnan et al., 2009; Major et al., 2007; Schneider et al., 1977). Estimated elimination half-lives ($t_{1/2}$; estimated $t_{1/2}$ values based on RDX concentrations in blood) indicate that RDX is more rapidly metabolized in mice than in rats and humans; estimated $t_{1/2}$ values were 1.2 hours for mice, 5–10 hours for rats, and 15–29 hours for humans (Sweeney et al., 2012b; Krishnan et al., 2009; Özhan et al., 2003; Woody et al., 1986; Schneider et al., 1977).

A more detailed summary of RDX toxicokinetics is provided in Appendix C, Section C.1.

1.1.3. Description of Toxicokinetic Models

A physiologically based pharmacokinetic (PBPK) model to simulate the pharmacokinetics of RDX in rats was first developed by Krishnan et al. (2009) and revised to extend the model to humans and mice (Sweeney et al., 2012a; Sweeney et al., 2012b). The Sweeney et al. (2012a) model consists of six main compartments: blood, brain, fat, liver, and lumped compartments for rapidly perfused tissues and slowly perfused tissues, and can simulate RDX exposures via the intravenous (i.v.) or oral route. This model assumes that the distribution of RDX to tissues is flow-limited, and

represents oral absorption as first-order uptake from the gastrointestinal (GI) tract into the liver, with 100% of the dose absorbed. RDX is assumed to be cleared by first-order metabolism in the liver. The model does not represent the kinetics of any RDX metabolites. The Sweeney et al. (2012a) and Sweeney et al. (2012b) PBPK models were evaluated and subsequently modified by the U.S. Environmental Protection Agency (EPA) for use in dose-response modeling in this assessment (see Appendix C, Section C.1.5).

1.2. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

In experimental animal studies, RDX test material administered in toxicology studies included formulations that ranged in purity (from 84 to 99.99%) and in particle size (from <66 to ~200 μm particle size). Differences in test material purity and particle size were taken into consideration while evaluating RDX toxicity findings; this is discussed in the literature search section and incorporated in the synthesis of evidence.

Mortality has been reported in the animal toxicology studies conducted for RDX. Due to the serious nature associated with a frank effect such as mortality, EPA specifically evaluated the database with respect to mortality (see Appendix C, Section C.3.1). In brief, mortality was observed following exposure to a range of doses in chronic-duration studies, in studies up to 6 months in duration, and during gestation (Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Cholakis et al., 1980; von Oettingen et al., 1949). In further analyzing the available evidence, mortality occurred at lower doses in rats compared with mice and following gavage administration compared with dietary administration. Additionally, mortality occurred to a greater extent with administration of RDX in the form of relatively finer particle sizes, likely due to faster dissolution of RDX leading to higher blood concentrations. Some investigators attributed the mortality to RDX-related cancer or noncancer effects (e.g., kidney or nervous system effects); others identified no cause for the animal deaths. Typically, evidence related to various hazards is presented and synthesized in distinct organ- or system-specific sections. However, in this case, the assessment does not present mortality in a hazard section by itself due to the likelihood that events leading to mortality fall under other specific hazards. Mortality evidence is considered in discussions of the evidence for organ/system-specific hazards where applicable.

1.2.1. Nervous System Effects

In humans, nervous system effects following RDX exposure have been observed in multiple case reports, and the association between RDX exposure and neurobehavioral effects has been examined in a single cross-sectional occupational epidemiology study. Information relevant to an examination of the association between RDX exposure and nervous system effects also comes from experimental animal studies involving chronic, subchronic, and gestational exposure to ingested RDX. No developmental neurotoxicity studies were available and minimal information was available to evaluate potential cognitive or behavioral effects associated with RDX exposure. A

summary of nervous system effects associated with RDX exposure is presented in Tables 1-2 and 1-3 and Figure 1-1. Experimental animal studies are ordered in the evidence table and exposure-response array by duration of exposure and then species.

Observational Studies in Humans

In a cross-sectional study by Ma and Li (1993), neurobehavioral effects were evaluated in Chinese workers occupationally exposed to RDX. Memory retention and block design scores[10] were significantly lower among exposed workers (mean concentrations of RDX in two exposed groups: 0.407 and 0.672 mg/m^3) compared to unexposed workers from the same plant. However, no significant differences were observed between the groups on other neurobehavioral tests (e.g., simple and choice reaction times, and letter cancellation test) (see Table 1-2). This study did not consider potential confounders such as alcohol consumption or co-exposure to trinitrotoluene (TNT), and there was limited information characterizing exposure to RDX.

Case reports suggest an association between RDX exposure (via ingestion, inhalation, and possibly dermal exposure) and neurological effects (see Appendix C, Section C.2). Severe neurological disturbances include tonic-clonic seizures (formerly known as grand mal seizures) in factory workers (Testud et al., 1996a; Testud et al., 1996b; Kaplan et al., 1965; Barsotti and Crotti, 1949), seizures and convulsions in exposed soldiers serving in Vietnam (Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968), seizures, dizziness, headache, and nausea following nonwartime/nonoccupational exposures (Kasuske et al., 2009; Davies et al., 2007; Küçükardali et al., 2003; Hett and Fichtner, 2002; Harrell-Bruder and Hutchins, 1995; Goldberg et al., 1992), and seizures in a child following ingestion of plasticized RDX from the mother's clothing (Woody et al., 1986).

Studies in Experimental Animals

Nervous system effects in experimental animals include an array of behavioral changes consistent with the induction of seizures by RDX exposure, and have been observed in the majority of chronic, subchronic, and developmental studies examining oral exposure to RDX (see Table 1-3 and Figure 1-1). Although study authors interchangeably used the terms seizures and convulsions, seizures, which result from abnormal electrical activity in the brain, can outwardly manifest in a variety of ways, including as convulsions. However, seizures can also manifest as facial twitches or tremors, or more subtly as increased irritability or aggression, absence of response to external stimuli, or they may go unnoticed. While behavioral methods exist to capture a spectrum of responses known to occur as a result of this aberrant neuronal activity, the most reliable detection

[10]The memory quotient index measured short-term hearing memory, visual memory, combined hearing and visual memory, and learning ability. The block design index measured visual perception and design replication, and the ability to analyze spatial relationships.

methods are electrophysiological (Racine, 1972). Only one acute exposure study, testing a single, high dose of RDX, included electrophysiologic recordings (Williams et al., 2011).

Convulsions (a sudden and irregular movement of a limb or of the body) have been reported in studies with different animal species and experimental designs. In every study that reported convulsions, the incidence of convulsions increased with dose. In 2-year dietary studies in rats (F344 and Sprague-Dawley) and mice ($B6C3F_1$), convulsions were observed beginning at doses of 35–40 mg/kg-day, but not at lower doses (Lish et al., 1984; Levine et al., 1983; Hart, 1976).[11] Subchronic dietary exposure to RDX was also associated with convulsions in the rat, although doses reported to increase convulsive activity were inconsistent across studies. Convulsions were reported in RDX-exposed rats at subchronic doses as low as 8 and 25 mg/kg-day (Crouse et al., 2006; von Oettingen et al., 1949). In three other studies of non-pregnant, adult rats involving exposure durations of 30–90 days, no evidence of seizures, convulsions, or tremors was reported at doses ranging from 1 to 50 mg/kg-day (MacPhail et al., 1985; Cholakis et al., 1980) (both unpublished technical reports).[12] Levine et al. (1990) reported convulsions in rats following subchronic exposure only at a dose of 600 mg/kg-day (a dose associated with 100% mortality); however, the unpublished technical report of this study (Levine et al., 1981a) reported convulsions at 600 and ≥30 mg/kg-day, thereby reducing confidence in the identification of the dose level at which nervous system effects were observed in this study. RDX exposure (by gavage) during gestation in the rat was associated with induction of seizures or convulsions in the dams at doses ranging from 2 to 40 mg/kg-day (Angerhofer et al., 1986; Cholakis et al., 1980) (unpublished technical reports), demonstrating that effects on the nervous system can be observed following exposure durations as short as 10–14 days. Convulsions were also reported in dogs exposed to 50 mg/kg-day RDX for 6 weeks (von Oettingen et al., 1949), but not 10 mg/kg-day for 13 weeks (Hart, 1974) (unpublished technical report); however, five of six monkeys exhibited convulsions following a gavage dose of 10 mg/kg-day for 13 weeks (Martin and Hart, 1974) (unpublished technical report). Linkage of these convulsions to seizure activity was most directly demonstrated by Williams et al. (2011), who observed abnormal electroencephalogram (EEG) activity consistent with seizure activity that coincided with physical manifestations ranging from subtle convulsive

[11]The 2-year dietary studies in F344 rats by Levine et al. (1983) and $B6C3F_1$ mice by Lish et al. (1984) were available only as a laboratory reports. An external peer review was sought by EPA in July 2012 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. A report of this peer review organized by Versar, Inc. is available on the Health and Environmental Research Online (HERO) database (U.S. EPA, 2012c). The 2-year dietary study in Sprague-Dawley rats by Hart (1976) is available as an unpublished technical report.

[12]The series of nine toxicology studies reported in Cholakis et al. (1980) were available only as a laboratory reports. An external peer review was sought by EPA in 2017 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented in six of the nine studies (90-day toxicity study in rats, initial 90-day toxicity study in mice, supplemental 90-day toxicity study in mice, teratology study in rats, teratology study in rabbits, and two-generation reproductive toxicity study in rats). A report of this peer review organized by Eastern Research Group, Inc. is available on the HERO database (U.S. EPA, 2017).

behaviors (e.g., twitches) to tonic-clonic seizures in rats acutely exposed to 75 mg/kg-day RDX via gavage.

In the only study addressing susceptibility to seizures (chemicals that may alter seizure frequency, severity, duration, or threshold), Burdette et al. (1988) found that seizure occurrence was more frequent in Long Evans rats exposed to a single dose of 50 or 60 mg/kg RDX by gavage when challenged with an audiogenic stimulus 8 and 16 hours after treatment. (Note: some uncertainty exists regarding the administered dose as neither the purity nor the specific particle size of the RDX used in the experiments by Burdette et al. (1988) was reported.) No audiogenic seizures were observed at the earlier 2- and 4-hour post-dosing test periods even though RDX plasma concentrations were elevated throughout the testing period, which could suggest that the blockade of GABAergic signaling by RDX (see Mechanistic Evidence section) needs to be sustained for some minimal duration to induce these types of effects. In a complementary experiment, Long Evans rats treated daily with 6 mg/kg-day RDX for up to 18 days required fewer stimulation trials compared to animals not treated with RDX to exhibit amygdaloid kindled seizures compared to controls. These findings provide evidence that RDX exposure can reduce the seizure threshold for other pro-convulsant stimuli, an adverse effect (U.S. EPA, 1998).

The majority of animal studies reported convulsions and/or seizures as clinical observations; thus, interpretation of these observations is limited because the nature and severity of convulsions and seizures were not more fully characterized. The 90-day study by Crouse et al. (2006)[13] was one of the few studies that collected and reported incidence data for convulsions and tremors, and demonstrated a clear dose-related increase in convulsions and tremors in male and female F344 rats associated with RDX exposure via gavage (see Table 1-3). Tremors were reported following administration of ≥12 mg/kg-day, persisting throughout the 90-day study. Convulsions were observed at ≥8 mg/kg-day in male and female rats; information on convulsion duration and onset after the start of dosing was not reported (Crouse et al., 2006).

In general, gavage dosing induced convulsions at lower doses than did dietary administration. For example, in the subchronic gavage study by Crouse et al. (2006) and the developmental gavage study by Cholakis et al. (1980), convulsions were observed in 1–3 F344 rats/group at doses of 2–8 mg/kg-day; at doses of 15–20 mg/kg-day, convulsions were observed in approximately 60–70% of the animals. Consistent with this pattern, even an acute (single dosing) gavage study reported seizures in 2/10 rats shortly after exposure to 12.5 mg/kg-day, and approximately 80% of rats developed spontaneous seizures shortly after exposure to 25–50 mg/kg-day (Burdette et al., 1988); the longevity of the seizure behaviors was also highly dose-dependent. In contrast, in a 2-year dietary study by Levine et al. (1983), convulsions were reported

[13]The 13-week gavage study in F344 rats by Crouse et al. (2006) was available only as a laboratory report. An external peer review was organized by Versar, Inc. in July 2012 to evaluate the accuracy of experimental procedures, results, and interpretation and discussion of the findings presented. The U.S. EPA (2012c) report of this peer review is available on the HERO database.

only at a dose of 40 mg/kg-day; no convulsions were observed at lower doses (≤8 mg/kg-day). The difference in response between gavage and dietary administration may be due to the bolus dosing resulting from gavage administration and the comparatively faster absorption and higher peak blood concentrations of RDX.

Several experimental animal studies documented that unscheduled deaths were frequently preceded by convulsions or seizures. In a 2-year study in rats, Levine et al. (1983) noted that tremors and/or convulsions were often seen in high-dose animals prior to their death. In a rat developmental toxicity study (Cholakis et al., 1980), investigators concluded that early deaths in dams were preceded by convulsions based on the observation of convulsions in one rat prior to death, and a similar appearance (e.g., dried blood around the mouth and nose) in other dams that died during the study. Convulsions preceding death were also observed in pregnant Sprague-Dawley rats exposed to RDX during gestation (Angerhofer et al., 1986). (Burdette et al., 1988) reported that 9/28 rats died during spontaneous seizure within 8 hours of administration (by gavage) of a single dose of 50 or 60 mg/kg RDX.

The 90-day Crouse et al. (2006) study provides the most detailed information on the relationship between convulsions and mortality (see Appendix C, Table C-10 for additional information on evidence of mortality associated with RDX exposure). Convulsions (3/20) and pre-term deaths (2/20)[14] were observed in male and female rats exposed to 8 mg/kg-day RDX; the incidences of both convulsions and pre-term deaths were higher in dose groups with greater exposures. Investigators stated that nearly all observed pre-term deaths in rats exposed to the three higher doses (10, 12, and 15 mg/kg-day RDX) for 90 days were preceded by neurotoxic signs such as rearing behavior, tremors and convulsions; however, pre-term death did not occur in all animals that convulsed. Convulsions were not typically observed during a functional observational battery (FOB) test conducted after exposure, possibly due to the time needed to complete exposures prior to beginning behavioral testing (convulsions typically occurred shortly after dosing). Of the 100 RDX-treated rats in the Crouse et al. (2006) study, convulsions were documented in 34 male and female rats across the five dose groups (with convulsions initially observed anywhere from day 7 to 87); based on additional information provided as a memorandum by study investigators (Johnson, 2015a), 26 of these 34 rats (76%) survived to the end of the 90-day study. In general, higher doses of RDX were associated with fewer days of exposure before the first convulsion was observed. Of the eight rats that exhibited convulsions prior to pre-term death, convulsions were documented anywhere from the same day that the animal died to 8 weeks prior to death. Of the 26 rats that seized and survived to day 90, the first seizures were observed as early as day 10 and as late as day 87. Thus, while an increase in mortality was observed in the Crouse et al. (2006) study at the same dose as convulsions, the additional information provided by Johnson (2015a) does not show as clear a correspondence between convulsions (and other neurotoxic

[14]At the 8 mg/kg-day dose level, the three rats that convulsed survived to the end of the study; no convulsions were observed in the two rats that died before study termination.

signs) and mortality. Analysis of these data is limited to the extent that convulsions may have occurred at times when animals were not observed and therefore may be undercounted in the individual animal data; however, Johnson (2015a) noted that it is unlikely that seizure observations were missed, since seizures generally occurred soon after dosing.

A few studies reported mortality that was not specifically associated with neurological effects (see Appendix C, Table C-10) (Angerhofer et al., 1986; Levine et al., 1981a; von Oettingen et al., 1949); however, in these studies, animals may not have been monitored for clinical observations or monitored with sufficient frequency to have observed convulsive activity prior to death. There were no reports of mortality subsequent to convulsions in case reports of nervous system effects in workers exposed to RDX during manufacture and in individuals exposed acutely as a result of accidental or intentional ingestion (see Appendix C, Section C.2).

Additional neurobehavioral effects associated with RDX exposure in rats included increased hyperactivity, hyper-reactivity to approach, fighting, and irritability at doses similar to those that induced tremors, convulsions, and seizures (20–100 mg/kg-day) (Levine et al., 1990; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a, b; von Oettingen et al., 1949). Hyperactivity and nervousness were also reported in male mice that received a subchronic exposure to 320 mg/kg-day RDX (Cholakis et al., 1980). No changes in motor activity, flavor aversion, scheduled-controlled behavior, or acoustic startle response were observed in a 30-day gavage study in rats at relatively low dose levels (≤10 mg/kg-day), although changes in acoustic startle response in acute exposures at higher doses (12.5–50 mg/kg) were noted (MacPhail et al., 1985). No significant changes in behavioral or neuromuscular activity were observed in rats following exposure to ≤15 mg/kg-day for 90 days (Crouse et al., 2006). Crouse et al. (2006) observed that stained haircoats and increased barbering in female F344 rats receiving 15 mg/kg-day may have been caused by the oral dosing procedure (gavage) alone.

Changes in absolute and relative brain weight were mixed across studies, and no studies included histopathologic evaluation of neuronal damage. Elevated absolute brain weights were reported in subchronic assays in B6C3F$_1$ mice and F344 rats (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a, b; Cholakis et al., 1980); however, the changes were not consistently observed across studies. Relative brain weights in some studies showed correspondingly greater increases compared to absolute brain weight (Crouse et al., 2006; Levine et al., 1983; Cholakis et al., 1980), but these changes were likely a result of changes in body weight in the study, and were not as useful a measure of effects of RDX on brain weights as absolute brain weight. In 2-year oral studies, a decrease in absolute brain weight of female B6C3F$_1$ mice (3–4% relative to control) was reported at doses ≥35 mg/kg-day (Lish et al., 1984), whereas an increase in absolute brain weight (2% relative to control) was observed in F344 rats at a dose of 40 mg/kg-day (Levine et al., 1983). Less emphasis is placed on evidence of organ weight changes from chronic (2-year) studies because normal physiological changes associated with aging and intercurrent disease may contribute to inter-animal variability that could confound organ weight interpretation (Sellers et al., 2007).

In some studies, seizures appeared soon after dosing, suggesting that seizure induction was more strongly correlated with dose level than with duration of exposure. Consistent with this observation are the findings of Williams et al. (2011), who demonstrated that RDX is rapidly absorbed and crosses the blood:brain barrier following oral administration in rats, and that distribution of RDX (8 µg/g wet weight) to the brain correlated with seizure onset. However, the incomplete or slow reversibility of the blockade of GABA receptor signaling after removal of RDX in the in vitro study by Williams et al. (2011) suggests that some effects might persist without the continued presence of RDX in the brain, which could permit cumulative effects.

While a dose-response relationship was observed consistently within studies, a dose that induced convulsions in animals in one study did not necessarily induce convulsions at the same dose in another study. This lack of consistency may be attributed, at least in part, to differences in the purity or particle size of the test material across studies. Assuming that increased particle size (and the corresponding reduction in available surface area compared with smaller particle sizes) results in slowed absorption and distribution to the brain, studies that used a larger particle size may be expected to produce less neurotoxicity in test animals. The mouse study by Cholakis et al. (1980) used a relatively large RDX particle size (200 µm) compared to the rat study by Levine et al. (1983) that used a smaller (<66 µm) particle size. This may explain why the Cholakis et al. (1980) subchronic dietary study in the mouse (doses up to 320 mg/kg-day RDX) and rat (doses up to 40 mg/kg-day) failed to report seizures or convulsions. Finally, differences in study design may have contributed to differences in reported neurological responses in subchronic and chronic duration studies. In particular, the protocols for observation for clinical signs (e.g., observations performed once daily in the morning in Levine et al. (1983)) may not have been sufficiently frequent to accurately measure the incidence of seizures or other nervous system effects.

The lack of developmental neurotoxicity studies was identified as a data gap within the available studies on RDX. A pilot study in rats did not directly investigate potential RDX nervous system effects but did find RDX in the brains of offspring rats as well as milk from dams treated with RDX during gestation (Hess-Ruth et al., 2007). Studies on chemicals with similar modes of action to RDX (e.g., bicuculline), combined with demonstrated transfer of RDX to perinatal rodent brains, suggest a potential for RDX to be harmful during brain development, possibly at a lower dose than required for neurotoxicity in adults. Further discussion on the potential developmental neurotoxicity of RDX can be found in Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes (see Section 1.3.3).

Table 1-2. Evidence pertaining to nervous system effects in humans

Reference and study design	Results			
Ma and Li (1993) (China) Cross-sectional study, 60 workers from the same plant exposed to RDX (30 in Group A [26 males; 4 females]; 30 in Group B [24 males; 6 females]), compared to 32 workers with similar age, education level, and length of employment from same plant with no exposure to RDX (27 males; 5 females). **Exposure measures**: Details of exposure measurement were not provided; two groups of workers exposed to the following mean RDX concentrations in air (basis for dividing workers into two exposure groups was not provided). **Concentration** (mg/m^3) (mean ± standard deviation): Group A 0.407 (± 0.332) Group B 0.672 (± 0.556) **Effect measures**[a]: Five neurobehavioral function tests and five additional memory subtests. **Analysis**: Variance (F-test); unadjusted linear regression, multiple regression, and correlation analysis.	**Neurobehavioral function tests, scaled scores** *(mean, standard deviation)*			
	Test	Control	Group A	Group B
	Memory retention*	111.3 (9.3)	96.9 (9.6)	91.1 (10.3)
	Simple reaction time (milliseconds)	493 (199)	539 (183)	578 (280)
	Choice reaction time (milliseconds)	763 (180)	775 (161)	770 (193)
	Block design* (elapsed time)	18.0 (5.4)	16.0 (4.3)	13.5(6.7)
	Letter cancellation (quality per unit time)	1,487 (343)	1,449 (331)	1,484 (443)
	*$p < 0.01$ (overall F-test); no statistically significant differences between Group A and Group B. Lower score indicates worse performance.			
	Memory retention subtests, scaled scores *(mean, standard deviation)*			
	Subtest	Control	Group A	Group B
	Directional memory*	23.5 (3.6)	17.2 (4.9)	18.1 (5.7)
	Associative learning*	24.9 (5.1)	20.0 (4.3)	18.5 (4.6)
	Image free recall*	24.1 (3.8)	20.9 (4.1)	20.4 (3.3)
	Recognition of nonsense pictures*	26.3 (3.6)	23.2 (4.9)	21.6 (4.3)
	Associative recall of portrait characteristics*	26.3 (3.3)	20.3 (4.4)	18.5 (4.3)
	*$p < 0.01$ (overall F-test); no statistically significant differences between Group A and Group B. Lower score indicates worse performance. Total behavioral score negatively correlated with exposure index (high exposure correlated with poor performance).			

[a]Symptom data were not included in evidence table because of incomplete reporting.

Table 1-3. Evidence pertaining to nervous system effects in animals

Reference and study design	Results
Convulsions and neurobehavioral effects	
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	One male in the 35 mg/kg-d dose group and one female in the 175/100 mg/kg-d group convulsed near the end of the study.
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	No nervous system effects, as evidenced by clinical signs or changes in appearance or behavior, were reported.
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Tremors, convulsions, and hyper-responsiveness to stimuli were noted in males and females at 40 mg/kg-d; no incidence data were reported.
Cholakis et al. (1980) Mice, B6C3F$_1$, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Hyperactivity and/or nervousness observed in 50% of the high-dose males; no signs observed in females[b]; no incidence data were reported.
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	No nervous system effects, as evidenced by clinical signs or changes in appearance or behavior, were reported.

Reference and study design	Results
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	No nervous system effects were reported.
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses: 0, 4, 8[b], 10, 12, 15 **Convulsions** *(incidence)* M: 0/10, 0/10, 1/10, 3/10, 8/10, 7/10 F: 0/10, 0/10, 2/10, 3/10, 5/10, 5/10 **Tremors** *(incidence)* M: 0/10, 0/10, 0/10, 0/10, 2/10, 3/10 F: 0/10, 0/10, 0/10, 0/10, 0/10, 1/10
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[c] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Hyper-reactivity to approach was observed in rats (sex not specified) receiving ≥100 mg/kg-d; no incidence data were reported. Tremors and convulsions were observed prior to death in one female and two male rats receiving 600 mg/kg-d.[d] (600 mg/kg-d was lethal to all rats.)
von Oettingen et al. (1949) Rats, sex/strain not specified, 20/group 90–97% pure, with 3–10% HMX; particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	Hyperirritability and convulsions were observed in the 25 and 50 mg/kg-d groups[b]; no incidence data were reported.

Reference and study design	Results				
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	No nervous system effects, as evidenced by clinical signs or changes in appearance or behavior, were reported.				
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus [e], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10[b]
	CNS effects characterized as depression, trembling, shaking, jerking, or convulsions *(incidence)*				
	M	0/3	0/3	0/3	2/3
	F	0/3	0/3	0/3	3/3
von Oettingen et al. (1949) Dogs, breed not specified, 5 females/group (control); 7 females/group (exposed) 90–97% pure, with 3–10% HMX; particle size not specified 0 or 50 mg/kg-d Diet 6 d/wk for 6 wks	Treated dogs exhibited convulsions, excitability, ataxia, and hyperactive reflexes[b]; no incidence data were reported.				
MacPhail et al. (1985) Rats, Sprague-Dawley derived CD, 8–10 males or females/group Purity 84 ± 4.7%; ≤66 µm particle size 0, 1, 3, or 10 mg/kg-d Gavage 30 d	No changes in motor activity, flavor aversion, scheduled-controlled response, or acoustic startle-response were reported.				
Cholakis et al. (1980) Rats, F344, 24–25 females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 6–19	Doses	0	0.2	2.0	20
	Convulsions *(incidence)*				
	F	0/24	0/24	1/24	18/25
Angerhofer et al. (1986) (range-finding study) Rats, Sprague-Dawley, 6 pregnant females/group Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 10, 20, 40, 80, or 120 mg/kg-d Gavage GDs 6–15	Convulsions preceding death were observed at ≥40 mg/kg-d; no incidence data were reported.				

Reference and study design	Results						
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 2, 6, or 20 mg/kg-d Gavage GDs 6–15	Convulsions and hyperactivity[b] were observed at 20 mg/kg-d; no incidence data were reported.						
Burdette et al. (1988) Rats, Long Evans, 10–21 males/group Exp 1: 0, 10, 20, or 60 mg/kg-d Exp 2: 0, 12.5, 25, or 50 mg/kg-d Experiments 1 and 2 conducted using the same study design, each with a control group Gavage (single exposure) 8-hr after exposure, rats placed in observation chamber; 0–64 kHz, 95 dB ultrasonic cleaner turned on for 1 min or until seizure initiated with uncontrolled running (whichever occurred first)	Doses	0	10	12.5	20	25	50	60
	Number of spontaneous seizures during 8-hr interval between dosing and audiogenic seizure testing (mean)							
	M	0	--	0.17 ± 0.2	--	1.4 ± 0.2*	4.5 ± 0.6*	--
	Note: first seizures in all 3 treatment groups observed within first 2 hrs after RDX exposure.							
	Prevalence of audiogenic seizures *(%)*[†]							
	M	0/31	1/10	0/10	3/10	4/10	10/12*	13/16*
	[†]Values estimated from graph using Grab It! Software and numbers of animals from Figure 2 of the paper. Statistical significance indicated by study authors; spontaneous seizures - $p < 0.012$; audiogenic seizures - $p < 0.017$.							
Brain weight								
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7	35	175/100		
	Absolute brain weight *(percent change compared to control)*							
	M	0%	−0.2%	0.61%	0.81%	−1%		
	F	0%	−2%	−2%	−4%*	−3%*		
	Relative brain weight *(percent change compared to control)*							
	M	0%	4%	2%	2%	5%		
	F	0%	−4%	−1%	−3%	18%*		
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8	40		
	Absolute brain weight *(percent change compared to control)*							
	M	0%	2%	−1%	2%	2%		
	F	0%	−0.3%	−0.4%	1%	2%*		
	Relative brain weight *(percent change compared to control)*							
	M	0%	0%	8%	2%	22%*		
	F	0%	−1%	3%	4%	20%*		

Reference and study design	Results						
Cholakis et al. (1980) Mice, B6C3F$_1$, 10−12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute brain weight *(percent change compared to control)*						
	M	0%	–	–	–	2%	2%
	F	0%	–	–	–	4%	2%
	Relative brain weight *(percent change compared to control)*						
	M	0%	–	–	–	6%	2%
	F	0%	–	–	–	0%	3%
Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Doses	0		80	160		320
	Absolute brain weight *(percent change compared to control)*						
	M	0%		0%	2%		10%
	F	0%		0%	4%		2%
	Relative brain weight *(percent change compared to control)*						
	M	0%		−3%	1%		8%
	F	0%		0%	3%		−4%
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute brain weight *(percent change compared to control)*						
	M	0%	–	–	–	3%	0%
	F	0%	–	–	–	0%	0%
	Relative brain weight *(percent change compared to control)*						
	M	0%	–	–	–	7%*	10%*
	F	0%	–	–	–	5%	6%
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Absolute brain weight *(percent change compared to control)*						
	M	0%	−1%	−0.3%	2%	5%*	7%*
	F	0%	−2%	6%	1%	4%	6%
	Relative brain weight *(percent change compared to control)*						
	M	0%	6%	10%	5%	3%	4%
	F	0%	−2%	−2%	−12%*	−12%*	−15%*

Reference and study design	Results						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[c] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Doses	0	10	30	100	300	600
	Absolute brain weight *(percent change compared to control)*						
	M	0%	1%	0.53%	−6%	–	–
	F	0%	−1%	1%	2%	–	–
	Relative brain weight *(percent change compared to control)*						
	M	0%	4%	7%	14%	–	–
	F	0%	0.3%	2%	5%	–	–

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Doses were calculated by the study authors.
[b]Mortality was reported in some RDX-treated groups in this study.
[c]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[d]Discrepancies in the doses at which convulsions occurred were identified in the technical report. The nervous system effects reported in this table and in the corresponding exposure-response array are those provided in the results section of the technical report (Levine et al., 1981a) and in the published paper (Levine et al., 1990). In other sections of the technical report, the authors reported that hyperactivity to approach and convulsions were observed in rats receiving ≥30 mg/kg-day (abstract and executive summary), or that mortality was observed in rats receiving 100 mg/kg-day and that hyperactivity to approach, tremors, and convulsions were observed in animals exposed to lethal doses (discussion).
[e]The species of monkey used in this study was inconsistently reported in the study as either Cynomolgus (in the methods section) or Rhesus (in the summary).

CNS = central nervous system; GD = gestational day; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

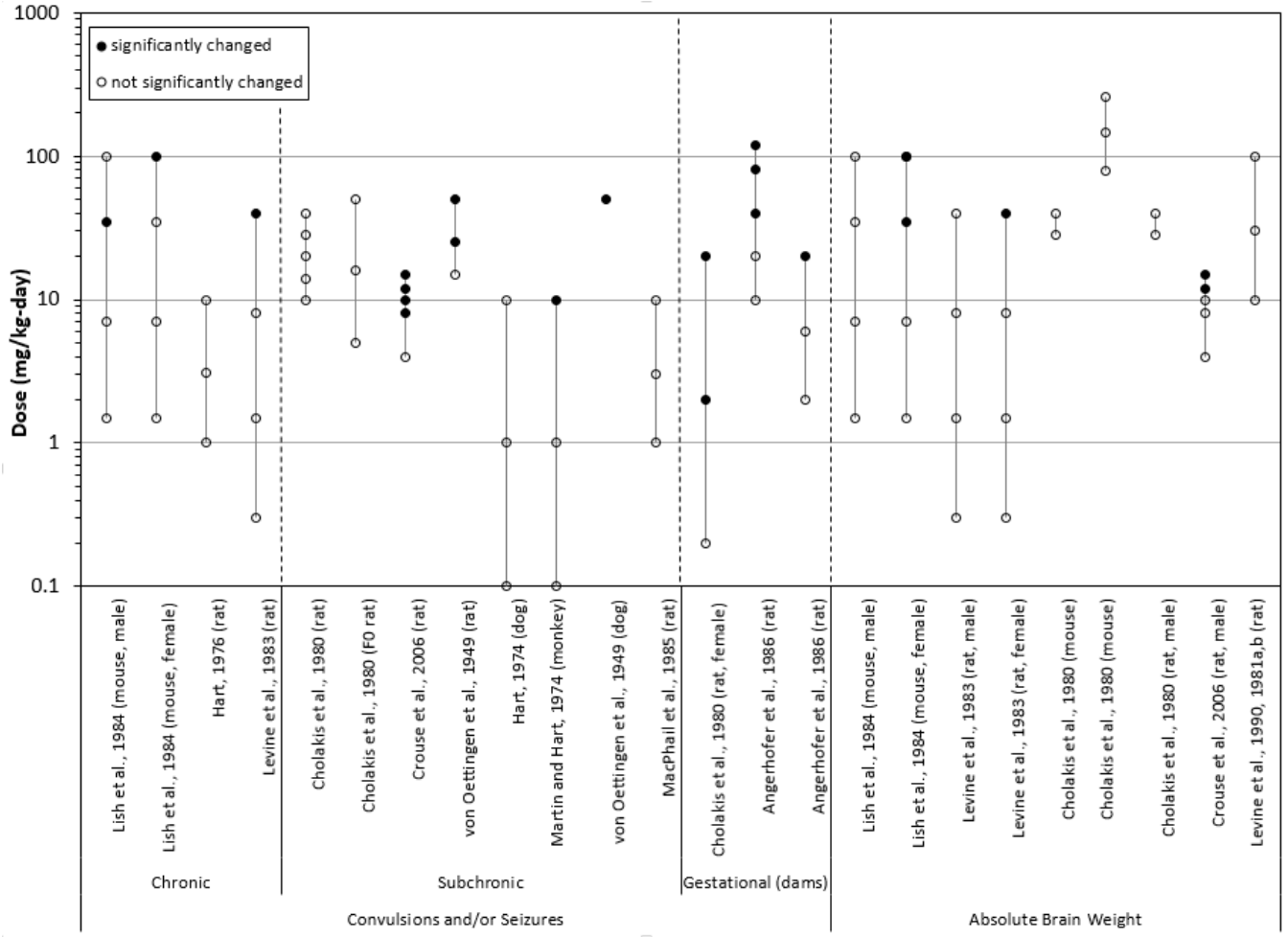


Figure 1-1. Exposure response array of nervous system effects following oral exposure.[a]

[a]Because convulsions and seizures are rare in experimental animals, any occurrence in an RDX-exposed group was considered treatment-related. Given the severity of this endpoint, a response in treated groups was determined to be significant (filled circles) in the array where there was any occurrence of convulsions and/or seizures reported in the study, whether or not the incidence was statistically significantly elevated over the control.

Mechanistic Evidence

Studies that have explored the mode of action (MOA) of RDX on the central nervous system (CNS) have focused on the potential impacts on neurotransmission. These studies implicate a MOA for RDX-induced seizures involving distribution of RDX from the blood to the brain (across the blood:brain barrier) and subsequent effects on neurotransmission, specifically gamma-amino butyric acid (GABA)-mediated signaling in limbic regions of the brain. There is significant evidence from the scientific literature to suggest that RDX neurotoxicity results from interactions of RDX with the $GABA_A$ receptor. GABA is a major inhibitory neurotransmitter in the brain, and the $GABA_A$ receptor has been implicated in susceptibility to seizures (Galanopoulou, 2008). A large literature base exists to support the relationship between blockade of $GABA_A$ergic neurotransmission and seizure induction, and $GABA_A$ergic pharmaceuticals are routinely used to suppress seizures in the treatment of epilepsy and other disorders (perhaps most recognizably, drugs in the benzodiazepine family).

In research conducted by the U.S. Army Center for Health Promotion and Preventative Medicine, Williams et al. (2011) and Bannon et al. (2009a) showed a correlation between blood and brain concentrations of RDX in rats that received a single oral dose of RDX (>98–99.5% purity) by gavage, which closely correlated with the time of seizure onset. RDX (75 mg/kg) was distributed to the brain in direct proportion to levels found in the blood, while time to seizure onset was reduced as RDX brain levels increased (Williams et al., 2011). Similarly, oral exposure to RDX (via a gel capsule: 3 or 18 mg/kg) resulted in quick absorption followed by transport to the brain and subsequent alterations in neurotransmission (Bannon et al., 2009a).

In receptor binding studies, RDX showed significant affinity for $GABA_A$ receptors (Williams et al., 2011; Williams and Bannon, 2009). Specifically, RDX showed an affinity for the picrotoxin convulsant site of the GABA channel, with nearly 100-fold less potency than picrotoxin itself. Consistent with the observations of abnormal electrical activity after in vivo RDX exposure (see discussion in previous section), in vitro RDX treatment of brain slices from the basolateral amygdala inhibited $GABA_A$-mediated inhibitory postsynaptic currents and initiated seizure-like electrical activity. Thus, RDX exposure appears to reduce the inhibitory effects of GABAergic neurons, resulting in a loss of inhibitory tone and enhanced excitability that can eventually lead to seizures (Williams et al., 2011; Williams and Bannon, 2009). The limbic system, and the amygdala and hippocampus in particular, are known to be critical to the development of seizures in various human conditions (e.g., epilepsy) and animal models (Jefferys et al., 2012; Gilbert, 1994). Consistent with the in vitro observations by Williams et al. (2011), Burdette et al. (1988) also implicated the limbic system in seizures caused by RDX exposure. Burdette et al. (1988) reported that the pattern of the seizure behaviors manifest in response to RDX exposure mimicked the sequence of behavioral stages observed following repeated electrical stimulation of temporal lobe structures by Racine (1972). In addition, amygdaloid kindled rats (rats subjected to patterns of electrical stimulation to this limbic region, which promotes the development of seizures) exhibited

pro-convulsant activity at a dose that was approximately half of the dose necessary for RDX to induce spontaneous seizures (rats treated with RDX also required fewer electrical stimulations to trigger kindled seizures) (Burdette et al., 1988). As these latter findings occurred at lower doses than RDX-induced increases in audiogenic seizures (Burdette et al., 1988), this further suggests a primary role for limbic regions (brain structures involved in sound-induced seizures may be indirectly affected). Potential limbic system involvement is also suggested given its role in integrating emotional and behavioral responses (including aggression) and the anecdotal observations of hyperactivity, hyper-responsiveness to approach, and irritability noted across several studies of RDX toxicity (Levine et al., 1990; Levine et al., 1983; Levine et al., 1981a, b; Cholakis et al., 1980; von Oettingen et al., 1949).

It is possible to construct a hypothetical MOA for RDX-induced seizure activity based on the evidence summarized above. These steps are consistent with ongoing efforts to identify an adverse outcome pathway (AOP) for ionotropic GABA receptor antagonism, reviewed in Gong et al. (2015) and Collier et al. (2016) and described in greater detail in the draft AOP available at https://aopwiki.org/. Following distribution of RDX to the brain:

1) Parent RDX acts as a $GABA_A$ receptor antagonist (supported by Schneider et al. (1977) and Williams et al. (2011)), binding noncompetitively to the picrotoxin convulsant site of the $GABA_A$ receptor (supported by Williams and Bannon (2009) and Williams et al. (2011)).

2) RDX binding to the $GABA_A$ receptor at the picrotoxin site blocks the conduction of chloride through the ion channel.

3) Reduced chloride conduction results in reduced GABA-mediated inhibition of neuronal signaling, often manifesting as a reduction in spontaneous inhibitory postsynaptic currents (sIPSCs). Williams et al. (2011) observed a reduction in the amplitude and frequency of sIPSCs in whole-cell in vitro recordings of neurons in brain slices from the rat basolateral amygdala after exposure to RDX. In addition, RDX treatment of slices inhibited GABA-induced currents.

4) Reduced inhibitory tone (e.g., reduced sIPSCs) increases the likelihood of action potentials by decreasing the resting potential of neuronal membranes (depolarization).

5) As a group of neurons begins firing abnormally and excessively (e.g., due to the reduced inhibitory tone, which typically would hyperpolarize, or reset, the membrane after firing), they can begin firing in a synchronized manner and initiate a wave of depolarization; these events can be detected electrophysiologically. Williams et al. (2011) observed a pattern of seizure-like neuronal discharges after in vivo RDX exposure and in vitro from slices of the basolateral amygdala in rats after adding RDX (the in vitro effects were not reversible after 40 minutes of washout).

The steps above provide a biologically plausible sequence of mechanistic events that result in the generation of seizure-like neuronal activity. Reduction of the inhibitory GABAergic signaling

is common to many convulsants, as summarized in Kalueff (2007). Some organochlorine insecticides, including alpha-endosulfan, dieldrin, and lindane, also exert neurotoxic effects through interaction with the $GABA_A$ receptor, and can produce a range of hyperexcitability effects (including convulsions) in mammals (Vale et al., 2003; Bloomquist, 1992; Suñol et al., 1989). The interaction of RDX with the $GABA_A$ receptor is directly supported by receptor-binding assays (Williams et al., 2011). Although these binding assays were performed on rat receptors, it is plausible that the results are relevant to human neurotoxicity. Seizures have been observed in many species, including humans, rats, mice, dogs, lizards, and birds at varying dosages and durations of exposure (Quinn et al., 2013; Mcfarland et al., 2009; Johnson et al., 2007; Bruchim et al., 2005; Küçükardali et al., 2003; Woody et al., 1986; Lish et al., 1984; Berry et al., 1983; Levine et al., 1983). A more recent meta-analysis of toxicogenomic data across a phylogenetically diverse set of organisms (rat, quail, fathead minnow, earthworm, and coral) demonstrated that neurotoxic responses are conserved in more highly-related species and that binding to the $GABA_A$ receptor is a common molecular initiating event (Garcia-Reyero et al., 2011). While these lines of evidence do not preclude a role of other receptors as yet unscreened for RDX binding affinity, they support a primary role for the GABAergic pathway described above in the development of RDX neurotoxicity.

As mentioned previously, the $GABA_A$ receptor is also a target of many anticonvulsant therapies (e.g., benzodiazepines, propofol, barbiturates) (Meldrum and Rogawski, 2007; Möhler, 2006). Additional support for the involvement of GABAergic signaling in the neurotoxicity of RDX comes from human case reports. In multiple case reports, medical intervention included treatment with benzodiazepines (commonly diazepam or lorazepam) to treat seizing patients (Kasuske et al., 2009; Davies et al., 2007; Küçükardali et al., 2003; Hett and Fichtner, 2002; Woody et al., 1986). Benzodiazepines act in large part by enhancing the effects of GABA at the $GABA_A$ receptor by increasing chloride conductance, resulting in anticonvulsant and relaxant effects (Goodman et al., 1996).

Some other pro-convulsant agents with minimal direct toxicity to nerve cells, such as sarin and some organophosphate pesticides, are known to act through inhibition of acetylcholinesterase (AChE) activity (McDonough and Shih, 1997). Some of the clinical signs observed following RDX exposure are similar to the clinical signs associated with organophosphate pesticides and nerve agents (Crouse et al., 2006; Burdette et al., 1988; Barsotti and Crotti, 1949). However, the limited data available for RDX do not support AChE inhibition as a contributing mechanism because: (1) blood and brain levels of AChE are unaffected by RDX (Williams et al., 2011; Williams and Bannon, 2009); and (2) in vitro neurotransmitter receptor binding studies do not reveal any affinity of RDX for acetylcholine receptors (Williams et al., 2011; Williams and Bannon, 2009). Additionally, common AChE-induced symptoms (salivation and lacrimation) have not routinely been observed (Williams et al., 2011). RDX showed no affinity for other receptors that are known targets of convulsants, including the glutamate family of receptors, nicotinic receptors, glycine receptors, and several monoamine receptors (Williams et al., 2011; Williams and Bannon, 2009).

In a microarray experiment, Bannon et al. (2009a) found that RDX caused a down regulation of an abundance of genes in the cerebral cortex related to neurotransmission, including those encoding proteins involved in synaptic transmission and vesicle transport. Genes encoding proteins involved in the glutamate pathway were also underexpressed, indicating a possible mechanism of action for RDX via excessive glutamate stimulation. The authors speculated that this depression of the major excitatory neurotransmitter system could be a negative response to the increase in seizure likelihood from RDX influx into the brain. Molecular changes in response to RDX have been described by Zhang and Pan (2009b), who observed significant changes in micro-RNA (miRNA) expression in the brains of B6C3F_1 mice fed 5 mg RDX/kg diet (estimated dose: 0.75–1.5 mg/kg-day; Bannon et al. (2009a)) for 28 days. One miRNA, miR-206, was upregulated 26-fold in RDX-exposed brains; brain-derived neurotrophic factor (BDNF) was identified as a downstream gene target of this miRNA, along with two other miRNAs that were upregulated in RDX-exposed brains (miR-30a and miR-195) (Zhang and Pan, 2009a, b). BDNF is a member of the neurotrophin family of growth factors, and promotes the survival and differentiation of existing and new neurons. Deng et al. (2014) conducted miRNA and mRNA profiling in rats to identify targets up or downregulated after 48-hour exposure to RDX, finding that many of the gene targets of these miRNAs were associated with nervous system function, and may contribute to the neurotoxicity of RDX. However, while effects of RDX on BDNF expression or other downstream targets may play a role in RDX neurotoxicity, the utility of miRNAs as predictors of toxicity has not been demonstrated and downstream targets of miRNA require verification (Bannon et al., 2009b). Despite this uncertainty, the potential for RDX exposure to modulate the expression or function of BDNF and other factors crucial to normal brain development raises concern for the possibility of neurotoxic effects with developmental exposure. Overall, the contribution, if any, of aberrant expression of a suite of miRNAs to the MOA for RDX neurotoxicity is unknown.

Some uncertainty remains regarding how the mechanistic understanding of RDX neurotoxicity may inform longer-term or cumulative exposures. To some extent, RDX binding at the picrotoxin convulsant site of the GABA channel may inform the relationship between exposure to the chemical and the time when a seizure is observed. Many of the available studies reported that seizures or convulsions were typically observed shortly after exposure, and several studies associated seizures with blood (and, correspondingly, brain) levels of RDX, indicating that a major contributing factor to the seizurogenic effects of RDX exposure appears to be the transient presence of RDX at target sites in the brain. Observations by Crouse et al. (2006), clarified in Johnson (2015a), showed that the median time to seizure after dosing in F344 rats is 55 minutes (range of 20–85 minutes); peak brain concentrations of RDX in F344 rats after single oral doses occurred within the first 3–4 hours after dosing (Bannon et al., 2009a). In addition, seizure intensity and the longevity of seizure-related behaviors was directly related to RDX dose, even with acute exposure (Burdette et al., 1988). These observations are all consistent with the presumed primary MOA. In general, across the RDX database, neurotoxicity, including induction of convulsions and seizures,

appears to be more strongly correlated with dose than duration of exposure. Crouse et al. (2006) reported that 80–90% of rats exposed to 12 and 15 mg/kg-day exhibited signs of neurotoxicity beginning on day 0 of the study, which continued for the study duration. However, some uncertainty remains. Gerkin et al. (2010) demonstrated that young C57/Bl6 mice injected intraperitoneally (i.p.) with picrotoxin to induce seizures had a significantly increased frequency of elevated neuronal activity ("Up state"), and firing rates were significantly increased in neocortical neurons up to 24 hours after exposure, despite the rapid clearance (within a few hours) of picrotoxin (Soto-Otero et al., 1989). It is possible that this extended period of elevated neuronal activity could increase the likelihood that a subsequent stimulus could trigger a seizure. While the study authors did not look at longer durations post exposure, these observations with picrotoxin may be consistent with the lack of complete reversibility of GABAergic signaling inhibition after removal of RDX in Williams et al. (2011). Thus, there remains the possibility that, in a chronic exposure scenario with repeated exposure to RDX and binding at the same site as picrotoxin, a generalized increase in elevated neuronal activity could increase the likelihood of seizures developing over time, or have other longer-term effects on normal brain function. While duration of exposure alone generally did not appear to be predictive of seizures (e.g., in Crouse et al. (2006), of the rats that survived the 90-day study, the range of time to onset of first observed convulsion after gavage exposure to 10 mg/kg-day RDX was as early as day 7 and as late as day 87), exposure to higher doses of RDX was associated with fewer days of exposure before the first convulsion was observed. The variation in time between the start of the experiment and the onset to first seizure with increasing dose could simply reflect the increased probability of action potentials with greater decreases in inhibitory tone at higher doses; however, it may also indicate a cumulative component of RDX neurotoxicity not accounted for by currently available mechanistic understanding.

Recent research in experimental animals has provided greater insight to inform a mechanistic basis of RDX neurotoxicity. While other possible MOA(s) may contribute to the overall neurotoxicity of RDX, the demonstrated affinity of RDX for the $GABA_A$ receptor, evidence of supportive electrophysiological changes in vivo or with direct application of RDX, and toxicokinetic evidence of distribution of RDX to the brain provide a mechanistic basis for the association of seizures with exposure to RDX. This MOA is similar to other well-studied convulsants and relevant to humans. The available information supports that RDX-induced seizures and related behavioral effects likely result from inhibition of GABAergic signaling within limbic regions of the brain.

Integration of Nervous System Effects

Evidence for nervous system effects associated with exposure to RDX comes from studies in both humans and animals. One occupational study reported memory impairment and decrements in certain neurobehavioral tests in workers exposed to RDX compared to controls (Ma and Li, 1993), and human case reports provide other evidence of an association between acute RDX exposure and neurological effects. There was consistent evidence of neurotoxicity associated with exposure to RDX; 11 of 16 repeat-dose animal studies (of varying design) reported neurological

effects (some severe), including seizures, convulsions, tremors, hyperirritability, hyper-reactivity, and behavioral changes, associated with RDX exposure (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981b; Cholakis et al., 1980; von Oettingen et al., 1949). In most of these studies, the occurrence of neurological effects was dose-related. In those studies that found no evidence of RDX-associated neurotoxicity (MacPhail et al., 1985; Cholakis et al., 1980; Hart, 1976, 1974), differences in dosing, particle size, and purity of the RDX administered potentially account for the lack of effect. Seizures resulting from RDX exposure likely result from inhibition of GABAergic signaling due to the interaction of RDX with the $GABA_A$ receptor. The pro-convulsant effects of RDX exposure are specific to CNS toxicity, as supported by observations of aberrant brain electrical activity corresponding with physical seizure behaviors Williams et al. (2011), as well as evidence of decreases in the seizure threshold for other centrally acting convulsants, including amygdaloid kindling and audiogenic stimuli (Burdette et al., 1988).

Together, toxicological information in animals and humans, supported by toxicokinetic and mechanistic information, provides a coherent identification of nervous system effects as a human hazard of RDX exposure.

1.2.2. Urinary System (Kidney and Bladder) Effects

The association between RDX exposure and effects on clinical measures of kidney function was examined in one occupational epidemiology study. Case reports, involving accidental exposure to ingested or inhaled RDX, offer some information on the potential for acute exposure to RDX to affect the kidney in humans. Organ weight and histopathology findings from experimental animal studies involving subchronic and chronic exposure to ingested RDX also provide data relevant to an examination of the association between RDX exposure and urinary system (kidney and bladder) effects. A summary of these effects associated with RDX exposure is presented in Tables 1-4 to 1-7 and Figure 1-2. Experimental animal studies are ordered in the evidence table and exposure-response array by duration of exposure and then by species.

Human case reports of individuals accidently exposed to unknown amounts of RDX by ingestion or inhalation provide some evidence that RDX affects the kidney. Reported symptoms included decreased urine output (Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Merrill, 1968), blood in urine (Kasuske et al., 2009; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Merrill, 1968), proteinuria (Kasuske et al., 2009; Küçükardali et al., 2003; Ketel and Hughes, 1972; Hollander and Colbach, 1969; Merrill, 1968), glucosuria (Küçükardali et al., 2003), elevated blood urea nitrogen (BUN) levels (Hollander and Colbach, 1969; Merrill, 1968), and one case of acute renal failure requiring hemodialysis following accidental inhalation of RDX (Ketel and Hughes, 1972). In many of these case reports, renal parameters returned to normal within a few days following exposure. No changes in renal parameters were reported in other individuals exposed to unknown amounts of RDX (Stone et al., 1969; Kaplan et al., 1965). In a cross-sectional epidemiologic study of workers from five U.S. Army munitions plants (69 exposed to RDX alone and 24 exposed to RDX and octahydro-1,3,5,7-tetranitro-

1,3,5,7-tetrazocine (HMX); RDX exposure range: undetectable [<0.01 mg/m^3] to 1.6 mg/m^3), no statistically significant differences in BUN or total serum protein between nonexposed and RDX-exposed groups were observed (Hathaway and Buck, 1977) (Table 1-4). As it is a cross-sectional study, no information was provided on the length of employment or other proxies that could be used to indicate exposure duration or cumulative exposure.

Studies in experimental animals provide some evidence that RDX exposure is associated with urinary system effects (see Table 1-5 and Figure 1-2). The strongest evidence of effects on this organ system is the collection of histopathological changes, including increased incidences of kidney medullary papillary necrosis and pyelitis, uremic mineralization, and bladder distention and/or cystitis, observed in male F344 rats exposed to 40 mg/kg-day RDX in the diet for 12 months or longer (Levine et al., 1983). The incidences of urinary system changes were higher at 2 years than at 12 months, but the response at both time points was robust (e.g., incidence of medullary papillary necrosis in male 40-mg/kg-day rats: 15/29 at 12 months, 18/31 at 2 years)[15]. Renal effects were considered the principal cause of treatment-related morbidity and mortality in these high-dose males. Similar kidney lesions were not observed in male rats in any dose group at the 6-month interim sacrifice (see Tables 1-6 and 1-7). Histopathological changes reported in some male rats in the lower-dose groups (0.3, 1.5, and 8 mg/kg-day) after 2 years on study were not dose-related, few in number, and consistent with background changes seen in aged rats.

Results from Levine et al. (1983) demonstrate a marked sex difference in response to RDX urinary system toxicity; no kidney or urinary bladder changes were associated with RDX exposure in female rats. In addition, mice appear to be less sensitive to the urinary system effects of RDX than rats; the incidences of kidney histopathological changes in male and female B6C3F$_1$ mice exposed to RDX in the diet for 2 years at concentrations as high as 100 mg/kg-day were similar to controls (Lish et al., 1984).

Histopathological findings in the urinary system from other experimental animal studies are largely consistent with the 2-year findings from Levine et al. (1983) and (Lish et al., 1984), i.e., that kidney and urinary bladder system effects are generally observed after RDX exposures longer than 6 months in duration and at high doses (e.g., ≥40 mg/kg-day). Specifically, no pattern of histopathological changes in the kidney were reported in rats exposed to RDX for 13 weeks (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a, b; Cholakis et al., 1980), in a 2-year study in Sprague-Dawley rats or 13-week study in beagle dogs that used a maximum dose of 10 mg/kg-day (Hart, 1976, 1974), or in rabbits exposed dermally to a cumulative dose of 165 mg/kg RDX in dimethylsulfoxide (DMSO) received over a 4-week period (5 days/week) (McNamara et al., 1974). In fact, in the 13-week study in F344 rats (Levine et al., 1981a) conducted by the same investigators that conducted the 2-year study in the same strain (Levine et al., 1983), chronic nephropathy was

[15]Denominator represents scheduled sacrifice animals plus spontaneous deaths and moribund sacrifice animals.

observed in both control and treated animals with no evidence of a dose-related increase in incidence.

Evidence of kidney histopathological changes in RDX-exposed animals following an exposure duration of less than 6 months is limited to an increased incidence of tubular nephrosis observed in B6C3F$_1$ mice exposed for 13 weeks to 320 mg/kg-day RDX (Cholakis et al., 1980), a dose eightfold higher than the dose that produced kidney and urinary bladder pathology in rats after 2 years of exposure. Increased incidence of minimal to mild mineralization of the medulla was observed in male and female monkeys exposed to 10 mg/kg-day RDX for 90 days by gavage (Martin and Hart, 1974), but the study authors did not identify this as treatment related. Finally, in a 2-generation study, Cholakis et al. (1980) reported an increased incidence of renal tubular epithelial-lined cysts in the kidney cortex in F2-generation rats exposed to RDX at doses up to 16 mg/kg-day. Because F2 animals were exposed for a relatively short duration (during gestation and through weaning only), and because no histopathology was performed for the parental and F1 generations, the kidney findings from this 2-generation study are difficult to interpret.

Other kidney endpoints—serum chemistry parameters that may indicate changes in renal function and kidney weights—did not provide consistent evidence of treatment-related changes. Measurement of serum chemistry parameters (including BUN and uric acid) in studies of RDX in mice, rats, dogs, and monkeys (Crouse et al., 2008; Levine et al., 1990; Lish et al., 1984; Levine et al., 1981a, b; Cholakis et al., 1980; Hart, 1976, 1974; Martin and Hart, 1974) revealed variations (increases or decreases) from the respective control groups that were not dose-related. Kidney weights in subchronic oral toxicity studies in rats, dogs, and monkeys did not show a clear pattern of change associated with RDX exposure. Kidney weight changes were either not dose-related or were inconsistently increased or decreased across studies (see Table 1-5). Less emphasis is placed on evidence of organ weight changes from chronic (2-year) studies (Lish et al., 1984; Hart, 1976) because normal physiological changes associated with aging and intercurrent disease may contribute to inter-animal variability that could confound organ weight interpretation (Sellers et al., 2007).

Exposure to HMX, the major contaminant in many of the available RDX studies, was associated with histopathological changes in the kidney and alterations in renal function in female, but not male, rats fed doses ≥450 mg/kg-day HMX for 13 weeks (see the Integrated Risk Information System [IRIS] assessment of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine [HMX] at https://www.epa.gov/iris). No effects were observed at doses ≤115 mg/kg-day. Given the dose levels where HMX appears to exhibit toxicity and the percentage of HMX (up to 10%) present as an impurity in technical grade RDX that would result in HMX exposures ≤60 mg/kg-day in the studies of RDX toxicity, the contribution of HMX to the observed kidney toxicity in studies of RDX is expected to be negligible. Further, differences in the pattern of toxicity (i.e., kidney effects observed only in RDX-exposed males and HMX-exposed females) also suggest that HMX contaminants were not responsible for kidney effects in rats exposed to RDX.

Table 1-4. Evidence pertaining to kidney effects in humans

Reference and study design	Results			
Hathaway and Buck (1977) Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate). Analysis group: limited to whites; 69 workers exposed to RDX alone and 24 workers exposed to RDX and HMX, compared to 338 workers not exposed to RDX, HMX, or TNT. **Exposure measures**: Exposure determination based on job title and industrial hygiene evaluation; exposed subjects assigned to two groups: undetected (<LOD) or ≥0.01 mg/m^3 (mean for employees with exposures ≥LOD: 0.28 mg/m^3). **Effect measures**: Renal function tests (blood) **Analysis**: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and χ^2 tests for comparison of proportions).	**Renal function tests: mean** *(standard deviation not reported)*			
		RDX exposed males*		
Test	Referent (n = 237)	Undetected (<LOD) (n = 22)	>0.01 mg/m^3 (n = 45)
BUN	15.5	15.6	16.4
Total protein	7.2	7.2	7.3
		RDX exposed females*		
	Referent (n = 101)	Undetected (<LOD) (n = 1)	>0.01 mg/m^3 (n = 25)
BUN	13.2	8	12.6
Total protein	7.3	7.6	7.2
*Includes both workers exposed to RDX alone and RDX and HMX. No differences were statistically significant in men or women.				

LOD = limit of detection

Table 1-5. Evidence pertaining to urinary system (kidney and bladder) effects in animals

Reference and study design	Results
Histopathological lesions	
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2−98.7% pure, with 3−10% HMX as contaminant; 83−89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	The incidence of cytoplasmic vacuolization of renal tubules was greater for RDX-treated males than the control group males after 6 mo of treatment. However, at 12 and 24 mo of treatment, this lesion was observed as frequently in controls as males treated with RDX. There was no increase in incidence of this lesion in females at any time point.

Reference and study design	Results					
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Histopathological examination of kidney did not reveal any significant differences compared to controls; lesions observed were not attributed to RDX treatment; incidence data were reported only for control and 10 mg/kg-d groups.					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs Note: More detailed histopathological results, including interim sacrifice data at 6 and 12 mo, are provided in Tables 1-6 to 1-8.	Data for male rats sacrificed on schedule (SS) and those that died spontaneously or were sacrificed moribund (SDMS) (summarized below) were analyzed separately. There were no treatment-related changes in incidence of kidney or urinary bladder lesions in females.					
	Doses	0	0.3	1.5	8.0	40
	Kidney, medullary papillary necrosis; 24 mo *(incidence)*					
	(SS)	0/38	0/36	0/25	0/29	0/4
	(SDMS)	0/17	1/19	0/27	0/26	18/27*
	(Sum)	0/55	1/55	0/52	0/55	18/31*
	Kidney, suppurative pyelitis; 24 mo *(incidence)*					
	(SS)	0/38	0/36	0/25	0/29	0/4
	(SDMS)	0/17	1/19	0/27	1/26	5/27*
	(Sum)	0/55	1/55	0/52	1/55	5/31*
	Kidney, uremic mineralization; 24 mo *(incidence)*					
	(SS)	1/38	0/36	0/25	0/29	0/4
	(SDMS)	0/17	1/19	2/27	0/26	13/27
	(Sum)	1/55	1/55	2/52	0/55	13/31
	Urinary bladder, luminal distention; 24 mo *(incidence)*					
	(SS)	0/38	0/36	0/25	0/29	1/4*
	(SDMS)	0/16	2/19	1/27	3/22	24/28*
	(Sum)	0/54	2/55	1/52	3/51	25/32*
	Urinary bladder, cystitis hemorrhagic/suppurative; 24 mo *(incidence)*					
	(SS)	0/38	0/36	0/25	1/29	0/4
	(SDMS)	0/16	2/19	1/27	0/22	18/27*
	(Sum)	0/54	2/55	1/52	1/51	18/31*

Reference and study design	Results				
Cholakis et al. (1980) Mice, B6C3F$_1$, 10−12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 80, 60, 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Doses	0	80	160	320
	Tubular nephrosis *(incidence)*				
	M	0/10	–	–	4/9*
	F	0/11	–	–	1/11
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Histopathological examination of kidney did not reveal any significant differences compared to controls; incidence data were reported only for control and 40 mg/kg-d groups.				
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	Data were reported only for F2 generation controls and 5 and 16 mg/kg-d groups.				
	Doses	0	5	16	50
	Renal tubule cysts, cortex *(incidence)*				
	M	4/10	4/10	8/10	–
	F	3/10	4/10	8/10	–
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Histopathological examination of kidney did not reveal any significant differences compared to controls; incidence data were reported only for control and 15 mg/kg-d groups.				

Reference and study design	Results				
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Doses	0	10	30	100
	Nephropathy, chronic, unilateral *(incidence)*				
	M	7/30	0/10	2/10	1/10
	F	4/30	0/10	0/10	1/10
	Nephropathy, chronic, bilateral *(incidence)*				
	M	22/30	8/10	7/10	1/10
	F	13/30	2/10	5/10	1/10
	Microcretions, focal, unilateral *(incidence)*				
	M	0/30	0/10	0/10	0/10
	F	4/30	5/10	0/10	1/10
	Microcretions, focal, bilateral *(incidence)*				
	M	0/30	0/10	0/10	0/10
	F	21/30	4/10	8/10	6/10
	Note: Incidence data not presented for 300 and 600 mg/kg-day dose groups since all rats died by week 3 at these doses.				
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Histopathological examination of kidney did not reveal any significant differences compared to controls; incidences were reported only for control and 10 mg/kg-d groups.				
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus[c], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10
	Medulla; mineralization, minimal to mild *(incidence)*				
	M + F	0/6	1/6	0/6	4/6
	Dilated tubules, mild to moderate *(incidence)*				
	M + F	4/6	3/6	6/6	3/6
	Multinucleated cells, tubules, minimal to moderate *(incidence)*				
	M + F	5/6	0/6	3/6	6/6
	Eosinophilic inclusions, minimal to moderate *(incidence)*				
	M + F	2/6	0/6	0/6	3/6

Reference and study design	Results					
Kidney weight[d]						
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100
	Absolute kidney weight at 104 wks *(percent change compared to control)*					
	M	0%	−1%	4%	9%*	19%*
	F	0%	3%	1%	1%	−2%
	Relative kidney weight at 104 wks *(percent change compared to control)*					
	M	0%	3%	6%	11%*	27%*
	F	0%	1%	1%	2%	19%*
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Doses	0	1.0	3.1	10	
	Absolute kidney weight *(percent change compared to control)*					
	M	0%	−3%	−7%	2%	
	F	0%	14%	−4%	8%	
	Relative kidney weight *(percent change compared to control)*					
	M	0%	−1%	−4%	4%	
	F	0%	22%	3%	18%	
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Absolute kidney weight at 105 wks *(percent change compared to control)*					
	M	0%	2%	−7%	1%	0%
	F	0%	3%	3%	2%	2%
	Relative kidney weight at 105 wks *(percent change compared to control)*					
	M	0%	1%	0%	2%	20%*
	F	0%	3%	6%	5%	21%*

Reference and study design	Results						
Cholakis et al. (1980) Mice, B6C3F$_1$, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute kidney weight *(percent change compared to control)*						
	M	0%	–	–	–	18%	2%
	F	0%	–	–	–	–8%	–5%
	Relative kidney weight *(percent change compared to control)*						
	M	0%	–	–	–	29%	0%
	F	0%	–	–	–	–8%	–3%
Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Doses	0		80	160		320
	Absolute kidney weight *(percent change compared to control)*						
	M	0%		8%	11%		13%
	F	0%		–5%	–3%		0%
	Relative kidney weight *(percent change compared to control)*						
	M	0%		5%	9%		10%
	F	0%		–5%	–4%		–5%
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute kidney weight *(percent change compared to control)*						
	M	0%	–	–	–	–2%	–5%
	F	0%	–	–	–	1%	0%
	Relative kidney weight *(percent change compared to control)*						
	M	0%	–	–	–	1%	5%
	F	0%	–	–	–	6%	6%
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	Doses	0		5	16		50
	Absolute kidney weight *(percent change compared to control)*						
	M	0%		6%	–12%		–
	F	0%		–4%	–21%*		–

Reference and study design	Results						
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Absolute kidney weight *(percent change compared to control)*						
	M	0%	−3%	−4%	−1%	3%	5%
	F	0%	2%	5%	13%*	10%	15%*
	Relative kidney weight *(percent change compared to control)*						
	M	0%	3%	6%	2%	1%	3%
	F	0%	1%	−3%	−1%	−6%	−7%*
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Doses	0	10	30	100	300	600
	Absolute kidney weight *(percent change compared to control)*						
	M	0%	1%	1%	−9%	–	–
	F	0%	1%	3%	−1%	–	–
	Relative kidney weight *(percent change compared to control)*						
	M	0%	5%	7%	10%	–	–
	F	0%	3%	5%	2%	–	–
Hart (1974)[e] Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0		0.1	1		10
	Absolute kidney weight *(percent change compared to control)*						
	M	0%		–	–		38%
	F	0%		–	–		−18%

Reference and study design	Results				
Martin and Hart (1974)[e] Monkeys, Cynomolgus or Rhesus[e], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10
	Absolute kidney weight *(percent change compared to control)*				
	M + F	0%	−2%	−3%	4%

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[c]The species of monkey used in this study was inconsistently reported in the study as either Cynomolgus (in the methods section) or Rhesus (in the summary).
[d]An analysis by Craig et al. (2014) found a statistically significant correlation between absolute, but not relative, kidney weights and renal histopathology. Therefore, only absolute kidney weight data from RDX studies are presented in Figure 1-2.
[e]Kidney weight data from the Hart (1974) and Martin and Hart (1974) studies were considered less informative than other studies. Hart (1974) reported organ weight data for high-dose dogs (3/sex/group) only, and the kidney weights from Martin and Hart (1974) were highly variable across monkeys (e.g., kidney weights for the control animals ranged from 4.9 to 13.1 g). Therefore, kidney weight data from these two studies were not presented in the exposure-response array for urinary system effects (Figure 1-2).

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

SDMS =spontaneous death or moribund sacrifice; SS = scheduled sacrifice.

Table 1-6. Six-, 12-, and 24-month incidence of kidney endpoints in male F344 rats reported for statistical evaluation in Levine et al. (1983)

Doses (mg/kg-d)	0	0.3	1.5	8.0	40
Medullary papillary necrosis *(incidence)*					
6 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	–	–	0/5
Sum	0/10	0/10	0/10	0/10	0/15
12 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	0/3	–	15/19*
Sum	0/10	0/10	0/13	0/10	15/29*

Doses (mg/kg-d)	0	0.3	1.5	8.0	40
24 mo					
SS	0/38	0/36	0/25	0/29	0/4
SDMS	0/17	1/19	0/27	0/26	18/27*
Sum	0/55	1/55	0/52	0/55	18/31*
Pyelitis *(incidence)*					
6 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	–	–	0/5
Sum	0/10	0/10	0/10	0/10	0/15
12 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	0/3	–	1/19
Sum	0/10	0/10	0/13	0/10	1/29
24 mo					
SS	0/38	0/36	0/25	0/29	0/4
SDMS	0/17	1/19	0/27	1/26	5/27*
Sum	0/55	1/55	0/52	1/55	5/31*
Pyelonephritis *(incidence)*					
6 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	–	–	0/5
Sum	0/10	0/10	0/10	0/10	0/15
12 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	0/3	–	1/19
Sum	0/10	0/10	0/13	0/10	1/29

Doses (mg/kg-d)	0	0.3	1.5	8.0	40
24 mo					
SS	0/38	0/36	0/25	1/29	0/4
SDMS	0/17	0/19	2/27	1/26	1/27
Sum	0/55	0/55	2/52	2/55	1/31

*Statistically significant ($p < 0.05$) based on analysis by study authors.

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

SDMS = spontaneous death or moribund sacrifice; SS = scheduled sacrifice.

Source: Levine et al. (1983).

Table 1-7. Six-, 12-, and 24-month incidence of urinary bladder endpoints in male F344 rats reported for statistical evaluation in Levine et al. (1983)

Doses (mg/kg-d)	0	0.3	1.5	8.0	40
Luminal distention *(incidence)*					
6 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	–	–	0/5
Sum	0/10	0/10	0/10	0/10	0/15
12 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	0/3	–	18/19*
Sum	0/10	0/10	0/13	0/10	18/29
24 mo					
SS	0/38	0/36	0/25	0/29	1/4*
SDMS	0/16	2/19	1/27	3/22	24/28*
Sum	0/54	2/55	1/52	3/51	25/32*
Cystitis, hemorrhagic/suppurative *(incidence)*					
6 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	–	–	0/5
Sum	0/10	0/10	0/10	0/10	0/15
12 mo					
SS	0/10	0/10	0/10	0/10	0/10
SDMS	–	–	0/3	–	17/19*
Sum	0/10	0/10	0/13	0/10	17/29
24 mo					
SS	0/38	0/36	0/25	1/29	0/4
SDMS	0/16	2/19	1/27	0/22	18/27*
Sum	0/54	2/55	1/52	1/51	18/31*

*Statistically significant ($p < 0.05$) based on analysis by study authors.

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

SDMS = spontaneous death or moribund sacrifice; SS = scheduled sacrifice.

Source: Levine et al. (1983).

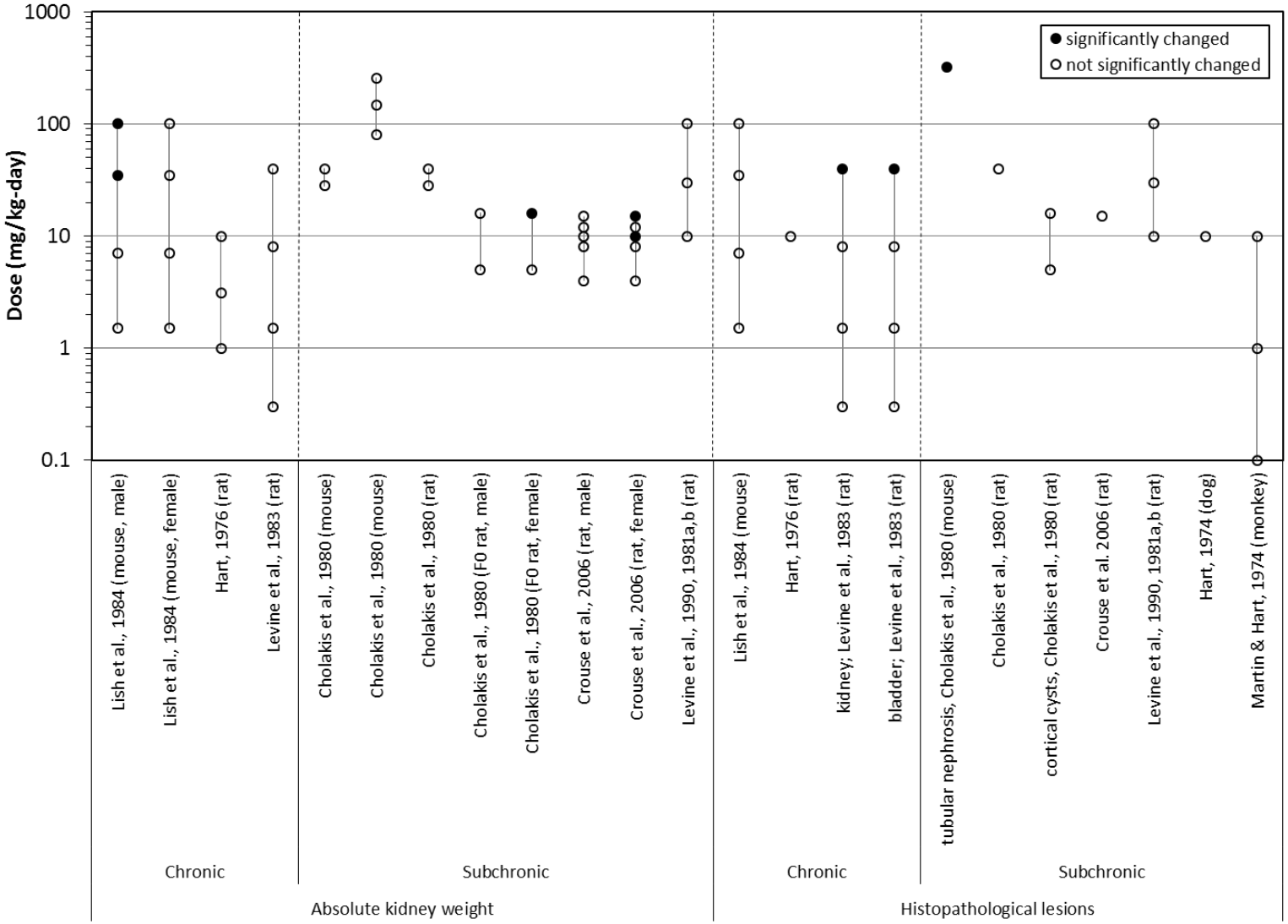


Figure 1-2. Exposure-response array of urinary system (kidney and bladder) effects.

Mechanistic Evidence

No MOA information is available for RDX-induced urinary system effects. Mechanistic information underlying the neurotoxicity observed with RDX exposure, and the specific affinity of RDX to the $GABA_A$ receptor-convulsant site (Williams et al., 2011; Williams and Bannon, 2009), suggests a biologically plausible role for the $GABA_A$ receptor in RDX-related effects on the urinary system.

GABA and GABA receptors have been identified in a number of peripheral tissues (Erdö et al., 1991; Ong and Kerr, 1990; Erdo, 1985). Brar et al. (2014) demonstrated that pretreatment with picrotoxin reduced the renoprotective effects of sodium valproate (which acts on both $GABA_A$ and $GABA_B$ receptors) in a rat model of ischemia-induced acute kidney injury, suggesting that $GABA_A$ receptors may be important in renal function. GABA is believed to play a role in the regulation of urination and bladder capacity (reviewed in Fowler et al. (2008) and Yoshimura and de Groat (1997)). In rats, injection of a $GABA_A$ receptor agonist inhibits the urination reflex (Igawa et al., 1993; Kontani et al., 1987). $GABA_A$ agonists injected into the periaqueductal gray area in rats inhibited reflex bladder activity, while injection of an antagonist reduced bladder capacity and increased the frequency of bladder reflex activity (Stone et al., 2011). RDX would be expected to act like an antagonist and increase bladder activity, although the impact of chronic exposure to RDX acting as a $GABA_A$ receptor antagonist is not known. Evidence of GABAergic signaling regulating bladder function, and the hypothesized disruption of that regulation by RDX via interaction with $GABA_A$ receptors, suggests a possible MOA for the kidney and urinary bladder lesions observed in particular by Levine et al. (1983); however, there does not appear to be any direct evidence (basic science or RDX-specific) to help discern the role of $GABA_A$ receptor in mediating these lesion types.

In summary, there are no studies available that inform mechanistically how RDX might lead to urinary system effects. There is evidence that RDX binds to $GABA_A$ receptors in neuronal tissues (Williams et al., 2011; Williams and Bannon, 2009), and it is biologically plausible that binding to the GABA receptor could occur in other tissues as well, contributing to the observed kidney and urinary bladder effects. However, the way(s) by which $GABA_A$ receptors may work in non-neuronal tissues and organs is not well understood, and the MOA by which RDX induces urinary system effects is not established.

Integration of Urinary System (Kidney and Bladder) Effects

Evidence for kidney effects resulting from RDX exposure consists of human case reports and findings of histopathological changes in rodents. In humans, evidence for kidney effects (including decreased urine output, blood in urine, and proteinuria) is limited to individuals with acute accidental exposure (ingestion and inhalation) to unknown amounts of RDX. No RDX-related changes in kidney parameters were found in a small cross-sectional study of RDX-exposed workers (Hathaway and Buck, 1977).

The 2-year Levine et al. (1983) study in F344 rats reported histopathological changes (papillary necrosis, pyelitis, luminal distension, and cystitis) in the kidney and urinary bladder in approximately 50% of male rats exposed to 40 mg/kg-day (the highest dose tested in this study), but only following exposure to RDX for longer than 6 months. Histopathological findings from other studies in rats, mice, and dogs (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a, b; Cholakis et al., 1980; Hart, 1976, 1974) are largely consistent with the 2-year findings from Levine et al. (1983), i.e., that kidney and urinary bladder effects are generally observed after RDX exposures longer than 6 months in duration and at high doses (e.g., ≥40 mg/kg-day). Other measures of kidney effects (kidney weights and serum chemistry parameters) did not provide consistent evidence of dose-related changes associated with RDX exposure.

Histopathologic findings from 2-year studies in F344 rats (Levine et al., 1983) and B6C3F$_1$ mice (Lish et al., 1984) provide evidence of sex and species differences in response to RDX. In contrast to the substantial urinary system toxicity observed in high-dose F344 male rats that was considered the primary cause of RDX-related morbidity and mortality (Levine et al., 1983), no kidney toxicity was associated with RDX in similarly-exposed female rats. Additionally, mice appear to be less sensitive than rats, based on an absence of RDX-related kidney histopathological changes in male and female B6C3F$_1$ mice exposed to RDX in the diet for 2 years at doses more than twofold greater than doses that produced substantial urinary system toxicity in male rats (Lish et al., 1984).

In light of the dose-related increase in histopathological changes in the kidney and urinary bladder in male rats in the Levine et al. (1983) study, and in particular the robust response in the high-dose animals, urinary system effects are a potential human hazard of RDX exposure.

1.2.3. Prostate Effects

No human studies were identified that evaluate the potential of RDX to cause effects on the prostate. There was limited information to evaluate prostate effects in animal studies, including two-year dietary studies in rats and mice (Lish et al., 1984; Levine et al., 1983), and one 90-day gavage study (Crouse et al., 2006). A summary of the prostate effects associated with RDX exposure in animals is presented in Tables 1-8 and 1-9 and Figure 1-3. Studies are ordered in the evidence tables and exposure-response arrays by duration of exposure and then by species.

The majority of animal studies available did not specifically evaluate whether there were prostate effects associated with RDX exposure. A significant, dose-related increase in the total incidence of suppurative prostatitis was reported in male F344 rats exposed to ≥1.5 mg/kg-day RDX in the diet for 2 years (Levine et al., 1983). Neither suppurative prostatitis nor any other treatment-related prostate effects were observed in a 2-year dietary study in mice (Lish et al., 1984). Suppurative prostatitis was not observed in 90-day studies in the rat involving oral (dietary or gavage) exposure to RDX (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a, b). In the 90-day gavage study (Crouse et al., 2006), mild subacute inflammation was observed in the prostate

of one of the rats at terminal sacrifice in the high dose group (15 mg/kg-day)[16]. The study authors considered the single observation to be consistent with expected background incidence and not treatment related. Excluding inflammation, there was little additional information to identify prostate effects associated with RDX exposure. Histopathological analysis identified a statistically significant increase in the incidence of spermatic granuloma of the prostate in rats fed 40 mg/kg-day RDX for up to 6 months. No gross abnormalities of the prostate were observed to accompany this finding, nor was this endpoint observed in 12- or 24-month dietary exposures to RDX (Levine et al., 1983).

Suppurative prostatitis is part of a continuum of inflammation. Further, suppurative prostatitis and non-suppurative prostatitis are not mutually exclusive; one form can evolve into another. Levine et al. (1983) also reported the incidence of non-suppurative (chronic-active) inflammation as well as subacute inflammation in male rats (see Table 1-8).

[16]A reporting discrepancy exists in Crouse et al. (2006) between the results section and the summary of histopathological findings in males in the appendix. The results section reports that mild subacute inflammation of the prostate was present in 1/7 males in the 15 mg/kg-day dose group at terminal sacrifice. The summary of histopathological findings (Appendix U) reports an incidence of 1/8 at 15 mg/kg-day.

Table 1-8. Two-year prostate inflammation incidence in male F344 rats Levine et al. (1983)

Doses (mg/kg-d)	0	0.3	1.5	8.0	40
Inflammation, subacute					
TS	1/38	0/36	0/25	0/29	0/4
SDMS	0/16	0/19	0/27	0/26	0/27
Sum	1/54	0/55	0/52	0/55	0/31
Inflammation, chronic-active					
TS	15/38	13/36	6/25	6/29	1/4
SDMS	5/16	5/19	7/27	5/26	1/27
Sum	20/54	18/55	13/52	11/55	2/31
Inflammation, suppurative					
TS	0/38	1/36	2/25*	4/29*	0/4
SDMS	2/16	3/19	7/27*	8/26	19/27*
Sum	2/54	4/55	9/52*	12/55*	19/31*
All inflammation					
Sum	23/54	22/55	21/52	23/55	21/31

*Statistically significant ($p < 0.05$) based on analysis by study authors.

SDMS = spontaneous death or moribund sacrifice; TS = terminal sacrifice.

Source: Levine et al. (1983)

As noted by the SAB in their review of the external review draft of the RDX assessment (SAB, 2017), the incidences of all observations of inflammation at 24 months in the Levine et al. (1983) study were similar in all dose group (approximately 40%) except for the high-dose group (68%). The incidence rate in the control and three lowest dose groups is lower than the background incidence of inflammation (70.4%) in a retrospective analysis of background lesions in male accessory sex organs of F344 rats reported by Suwa et al. (2001). The lower incidence in F344 rats reported in Levine et al. (1983) suggests there may have been differences in histopathological practices between those employed by Levine et al. (1983) and more recent diagnostic criteria. For example, inflammation incidence varies across lobes of the prostate and the methods reporting in Levine et al. (1983) does not provide sufficient information to determine how the prostates were evaluated for inflammation. Finally, male rats in the high-dose group (40 mg/kg-day) were moved from group to individual housing between weeks 30 to 40 during the study, due to a high incidence of fighting. The increased incidence of fighting may have contributed to conditions that lead to urogenital infections in male rats (Creasy et al., 2012).

The severity of inflammation differs in Levine et al. (1983) compared to that reported in Suwa et al. (2001). In reviewing the background incidence of all inflammation in the prostate in 1,768 F344 rats, Suwa et al. (2001) identified an average severity grade of "mild." In Levine et al. (1983), there is an increased incidence of suppurative prostatitis, which is more severe (characterized by the formation of pus and a high concentration of neutrophils). There was also a shift from chronic inflammation to suppurative inflammation with increasing dose of RDX starting at 1.5 mg/kg-day (see Table 1-8). At the highest dose, the shift from chronic to suppurative inflammation is clear, with only two animals exhibiting chronic inflammation and 19 identified as having suppurative inflammation.

Some reports have hypothesized that the observation of prostate inflammation in Levine et al. (1983) is secondary to a bacterial infection unrelated to RDX toxicity (ATSDR, 2012; Sweeney et al., 2012a; Crouse et al., 2006). For example, in describing the results from the 2-year dietary study in rats, Crouse et al. (2006) observed that the inflammation reflects a common condition in rodents, noting that since 85% of the incidence occurred in rats found at spontaneous death or moribund sacrifice (SDMS), it was most likely that the condition was a result of an incidental bacterial infection. Although the proportion of suppurative prostatitis was higher in SDMS rats, there was an increasing trend with dose in both the scheduled sacrifice (SS) and SDMS groups; the incidence of suppurative prostatitis in the control group was 4% when the SS and SDMS groups were combined. Additionally, the dose-related nature of the increased incidence suggests that the primary cause (potentially leading to bacterial infection) was treatment-related, as a more uniform distribution of rats with suppurative prostatitis would be expected with a spontaneous or age-related lesion. The dose-responsiveness could be explained if the infections were secondary to treatment-related immunotoxicity, but there is no evidence from Levine et al. (1983) to support this possibility. A more thorough analysis of immune endpoints in a 90-day gavage exposure of F344 rats did not identify any immunotoxic effects associated with RDX (Crouse et al., 2006). In general, causes of prostatitis other than infection exist, including stress, endocrine effects (i.e., changing prolactin levels), and autoimmune dysfunction (see, for example, Bosland, 1992; Gatebeck et al., 1987; Parker and Grabau, 1987).

Table 1-9. Evidence pertaining to prostate effects in animals

Reference and study design	Results					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Data for male rats sacrificed on schedule (SS) and those that died spontaneously or were sacrificed moribund (SDMS) (summarized below) were analyzed separately.					
		0	0.3	1.5	8.0	40
	Prostate, suppurative inflammation (prostatitis); 24 mo *(incidence)*					
	SS	0/38	1/36	2/25*	4/29*	0/4
	SDMS	2/16	3/19	7/27*	8/26	19/27*
	(Sum)	2/54	4/55	9/52*	12/55*	19/31*
	Spermatic granuloma of the prostate; 6 mo *(incidence)*					
	SS	0/10	2/10	2/10	1/10	6/10*
	SDMS	--	--	--	--	2/5
	(Sum)	0/10	2/10	2/10	1/10	8/15*
Lish et al. (1984) Mice, B6C3F1, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs		0	1.5	7.0	35	175/100
	Prostate, chronic inflammation; 24 mo *(incidence)*[a]					
	M	1/62	1/3	0/1	1/1	0/27
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Prostate, mild subacute inflammation *(incidence)*						
	M	0/10	–	–	–	–	1/8

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Examination only required by protocol in the control and high-dose groups.

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

SDMS = spontaneous death or moribund sacrifice; SS = scheduled sacrifice.

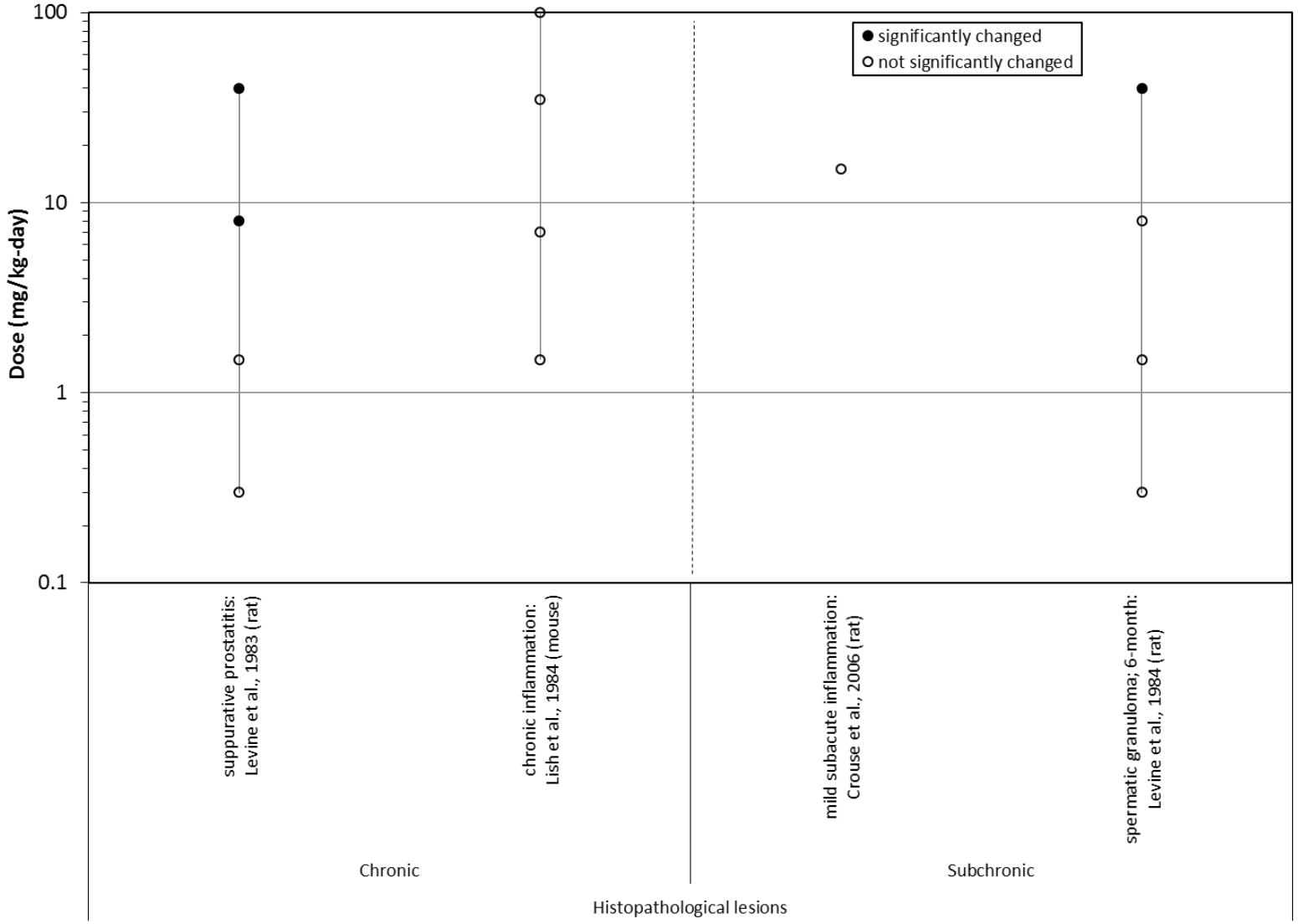


Figure 1-3. Exposure-response array of prostate effects.

Mechanistic Evidence

No MOA information is available for RDX-induced prostate effects. However, mechanistic information underlying the neurotoxicity observed with RDX exposure, and the specific affinity of RDX to the $GABA_A$ receptor-convulsant site (Williams et al., 2011; Williams and Bannon, 2009), suggests a biologically plausible role for the $GABA_A$ receptor in RDX-related effects, and provides some potential MOA hypotheses for the effects reported in Levine et al. (1983) that do not require bacterial infection.

One possibility is that effects would result from direct interactions with $GABA_A$ receptors located on the prostate. $GABA_A$ receptors have been identified on the prostate (Napoleone et al., 1990), providing a potential mechanism by which RDX could interact directly with the prostate. However, this would require that the prostate is actively maintained in a non-inflamed state, mediated by GABA; RDX binding to $GABA_A$ receptor-convulsant sites on the prostate would result in a reduction of the inhibitory effects of the GABA receptor, leading to increased inflammation (Johnson, 2015b). No evidence was found to support this potential pathway leading to prostate inflammation.

Another possibility is that alterations in hormonal signaling or circulating levels of estrogen or prolactin may lead to prostatitis. Prostate inflammation has been associated with endocrine disruptors in the environment (Cowin et al., 2010), and increased prolactin has been shown to cause lateral lobe prostatitis (Stoker et al., 1999b; Stoker et al., 1999a; Tangbanluekal and Robinette, 1993; Robinette, 1988). Typically, the inflammation seen is chronic and does not reverse over time (Robinette, 1988). Functional $GABA_A$ receptors have been identified in the anterior pituitary (Zemkova et al., 2008; Mayerhofer et al., 2001), which also serves as the primary source of prolactin. Thus, the prostate inflammation observed in the rat in the 2-year study by Levine et al. (1983) could have been produced by disruption of pituitary prolactin or another hormonal signal via interference with normal regulatory GABA-related hormonal control. However, no direct evidence for this hypothesized MOA is available. Levine et al. (1983) did not evaluate serum endocrine measures or pituitary weights, and pituitary adenomas that could account for higher prolactin levels were not observed.

Another hypothesis is that the prostate effects could be mediated through an autoimmune inflammatory response. $GABA_A$ receptor transcripts have been identified in immune cells of mouse models (Reyes-García et al., 2007; Tian et al., 2004), and $GABA_A$ receptor agonists have decreased cytotoxic immune responses and hypersensitivity reactions (Tian et al., 1999; Bergeret et al., 1998). In a mouse autoimmune model of multiple sclerosis, Bhat et al. (2010) found that treatment of macrophages challenged with lipopolysaccharide with various GABA agonists decreased cytokine production; addition of picrotoxin (which may have effects similar to those of RDX, as it binds to the same site) was able to reduce this effect. However, picrotoxin on its own did not significantly alter cytokine production, suggesting that the effects are limited to reversal of agonist-induced GABAergic activity (Johnson, 2015b). If an autoimmune mechanism was contributing to the effects

observed with RDX exposure, it is unclear why inflammation would be limited to the prostate. RDX has also tested negative in the only battery of immunotoxicity tests to which it was subjected (Crouse et al., 2006).

In summary, there are no studies available that inform mechanistically how RDX exposure might lead to prostate effects. There is evidence that RDX binds to $GABA_A$ receptors in neuronal tissues (Williams et al., 2011; Williams and Bannon, 2009), and it is biologically plausible that binding to the GABA receptor could occur in other tissues as well. Among the mechanistic information presented above, MOAs that require direct action on the prostate appear less likely; however, the ways that $GABA_A$ receptors work in non-neuronal tissues and organs is still not well understood, and the MOA by which RDX may induce prostate effects is unknown.

Integration of Prostate Effects

Suppurative prostatitis was reported in male F344 rats chronically exposed to RDX in the diet for 24 months (Levine et al., 1983). No other studies of equivalent duration were performed in rats to determine the consistency of this effect. Spermatic granuloma of the prostate was identified in F344 rats exposed to RDX for up to 6 months, but not at 12 or 24 months in the study; therefore, the biological significance of the 6-month finding is uncertain. A 24-month study in mice (Lish et al., 1984) did not report prostate effects associated with RDX exposure. No other animal studies of shorter duration identified prostate effects associated with RDX exposure. In light of the dose-related, statistically significant increase in suppurative prostatitis, there is suggestive evidence that prostate effects are a potential human hazard of RDX exposure.

1.2.4. Developmental Effects

No human studies were identified that evaluate the potential of RDX to cause developmental effects. Information relevant to an examination of the association between RDX exposure and developmental effects comes from a 2-generation reproductive toxicity study in rats and developmental studies in rats and rabbits involving oral administration of RDX during gestation. A summary of the developmental effects associated with RDX exposure is presented in Table 1-10 and Figure 1-4. Studies are ordered in the evidence tables and exposure-response arrays by duration of exposure and then by species.

Animal studies have reported decreases in offspring survival following administration of RDX. Pup survival rates in the F0 and F1 generations (including both stillborn pups and postnatal deaths through the age of weaning) were statistically significantly decreased in RDX-exposed CD rats compared to controls in the only available two-generation reproductive toxicity study of RDX (Cholakis et al., 1980). This observation was noted only at the highest dose tested (50 mg/kg-day) that also produced toxicity in adults (mortality [18%], reduced body weights [8–14%], and reduced food consumption [10–17%]). Decreased fetal viability was observed at the highest dose tested, 20 mg/kg-day, in a developmental toxicity study in F344 rats (Cholakis et al., 1980), although no effect on live fetuses was observed in a developmental toxicity study in Sprague-Dawley rats at the same

dose (Angerhofer et al., 1986); both of these studies reported significant mortality (29–31%) in dams at 20 mg/kg-day. Increased resorptions were similarly limited to the highest dose tested (20 mg/kg-day) (Cholakis et al., 1980). Both studies started treatment with RDX on gestational day (GD) 6, which may contribute to the incidence of resorptions observed in the control and treated groups. As noted in EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), treatment beginning around the time of implantation may result in an increase in implantation loss that reflects variability that is not treatment related. There was no evidence of maternal toxicity, embryotoxicity, or decreased fetal viability in a teratology study of pregnant New Zealand White (NZW) rabbits administered RDX by gavage from GD 7 to 29 at doses up to 20 mg/kg-day (Cholakis et al., 1980), suggesting that rabbits may be less sensitive to RDX toxicity than rats.

Statistically significant, dose-related reductions in fetal body weight and length were reported in Sprague-Dawley rats administered RDX by gavage from GD 6 to 15 (Angerhofer et al., 1986).[17] Decreased fetal body weight (9%) and body length (5%), with statistically significant trends, were observed at 20 mg/kg-day, a dose that produced significant (31%) mortality in the dams. A similar reduction in fetal body weight of 7% (not statistically significant) was observed in F344 rats exposed to RDX at 20 mg/kg-day, a dose associated with 29% maternal mortality (Cholakis et al., 1980). Dose-related reductions in fetal body weight were not observed in NZW rabbits at doses up to 20 mg/kg-day (Cholakis et al., 1980).

No treatment-related effects on morphological development have been reported in rats exposed to a dose as high as 20 mg/kg-day RDX, a dose that resulted in 29–31% maternal mortality (Angerhofer et al., 1986; Cholakis et al., 1980). Examination of rabbits administered RDX at doses up to 20 mg/kg-day from GD 7 to 29 also provided no evidence of treatment-related developmental anomalies (Cholakis et al., 1980). Although increased incidences of enlarged frontal fontanel and unossified sternebrae were observed in fetuses of all groups of NZW rabbits administered RDX (Cholakis et al., 1980), these developmental anomalies did not exhibit a dose-related increase in the number of either fetuses or litters affected, and were thus interpreted as not being treatment-related by the study authors (Cholakis et al., 1980). Neither individual litter data nor historical control data from the performing laboratory were available to assist in the interpretation of these findings. The author's interpretation is supported by the following additional considerations. A report of historical control incidences of fetal skeletal observations in NZW rabbits for 224 prenatal developmental toxicology studies conducted in 8 contract research laboratories during the period of 1988–1992 (MTA, 1992) included findings from 26,166 fetuses of 3,635 litters. Background control incidences of enlarged anterior fontanel were observed in 8 fetuses (0.031%) of 7 litters (0.193%), while sternebrae agenesis (which may not be entirely comparable to the finding of

[17]The statistical analyses presented by the study authors were performed on a per fetus basis; EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991) recommend that fetal data be analyzed on a per litter (rather than per fetus) basis. In a reanalysis of the Angerhofer et al. (1986) data by EPA on a per litter basis, fetal body weight and length showed statistically significant decreasing trends.

unossified sternebrae in Cholakis et al. (1980) was found in 10 fetuses (0.038%) of 5 litters (0.138%). Although the use of concurrent control data is preferable for the interpretation of developmental toxicity data, this historical information supports the low control incidences of these findings in the Cholakis et al. (1980) study as being within typical historical parameters. It is also noted that the non-dose-related pattern of increased enlarged fontanel and unossified sternebrae across treated groups in Cholakis et al. (1980) was similar to the pattern of decreases in fetal body weight in the same study, suggesting a possible link between these particular sternebral and fontanel anomalies with fetal growth status. Given the lack of dose-related increases in the incidences of these anomalies, and patterns that mirrored fetal body weight decreases (which were also not dose-related), the findings of enlarged frontal fontanel and unossified sternebrae were not considered treatment-related. Gestational administration of RDX to NZW rabbits did not result in any other dose- and treatment-related skeletal abnormalities.

Table 1-10. Evidence pertaining to developmental effects in animals

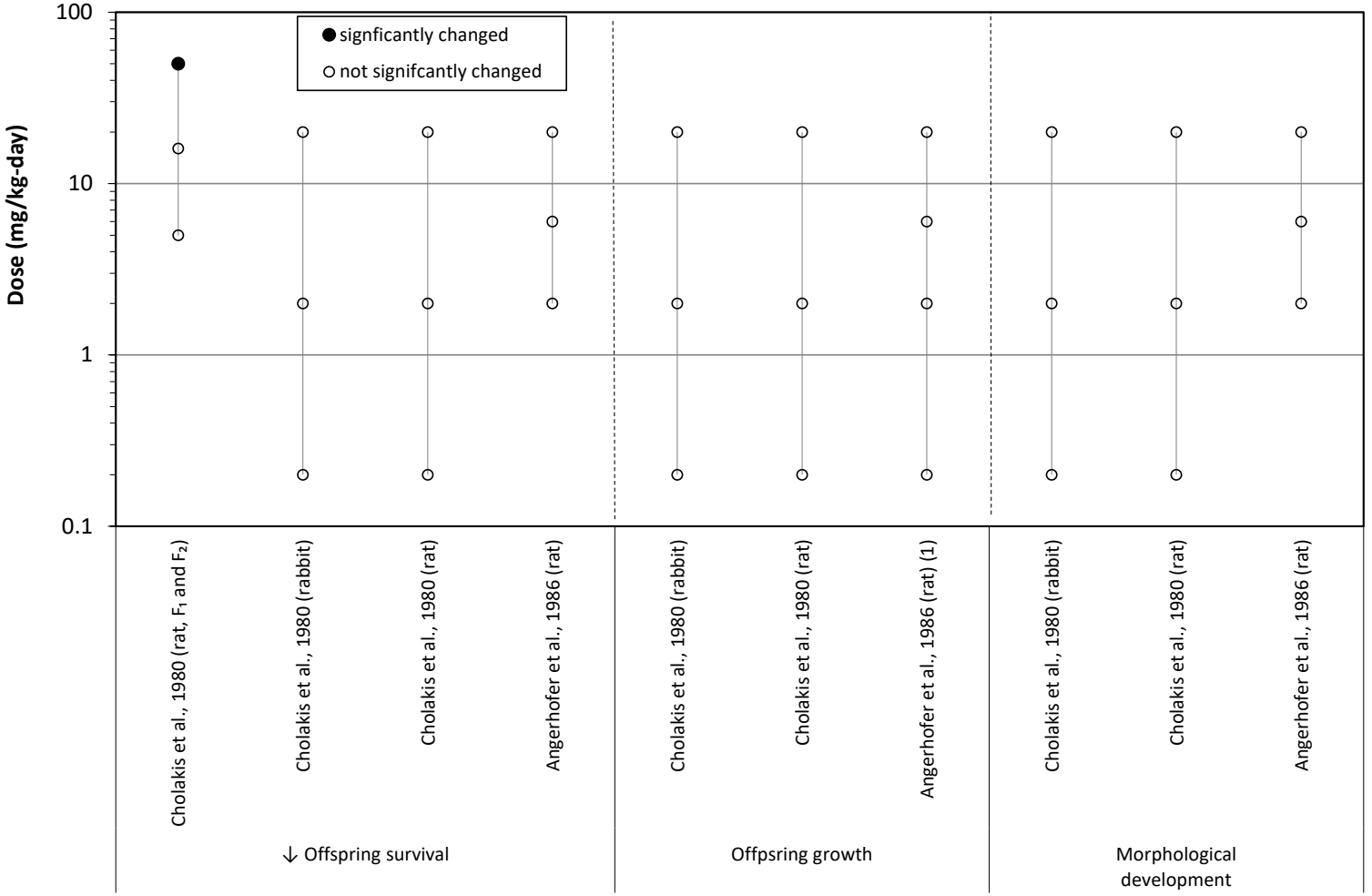
Reference and study design	Results				
Prenatal mortality/offspring survival					
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26 sex/group; F2: 10 sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	Doses	0	5	16	50
	Stillborn pups *(incidence)*				
	F1	8/207	6/296	4/259	16/92*
	F2	6/288	6/290	2/250	24/46*
	Offspring survival at birth *(percent of fetuses)*				
	F1	96%	98%	98%	83%*
	F2	98%	98%	99%	48%*
	Survival at weaning *(percent of liveborn pups)*				
	F1	87%	96%	90%	8%
	F2	79%	86%	79%	0%
	F0 maternal deaths occurred at 50 mg/kg-d. Only six F1 females in this group survived to serve as parental animals; none of the surviving six died during subsequent treatment. Note: results on a per litter basis were not provided.				
Cholakis et al. (1980) Rabbits, NZW, 11–12/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 7–29	Doses	0	0.2	2	20
	Early resorptions *(mean percent per dam)*				
		6%	5%	4%	1%
	Late resorptions *(mean percent per dam)*				
		8%	5%	3%	3%
	Viable fetuses *(mean percent per dam)*				
		85%	82%	77%	94%

Reference and study design	Results				
Cholakis et al. (1980) Rats, F344, 24–25 females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants. 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 6–19	Doses	0	0.2	2.0	20
	Early resorptions *(mean percent per dam)*				
		6.0%	2.5%	4.8%	15.3%
	Late resorptions *(mean percent per dam)*				
		0.5%	0.5%	0.3%	1.6%
	Complete litter resorptions *(number of litters)*				
		0	0	0	2
	Viable fetuses *(mean percent per dam)*				
		93.2%	97.6%	94.9%	81.4%
	Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d.				
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group) Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 2, 6, or 20 mg/kg-d Gavage GDs 6–15	Doses	0	2	6	20
	Resorptions *(percent of total implantations)*				
		4.8%	6.1%	5.9%	6.4%
	Early resorptions *(percent of total implantations)*				
		4.8%	6.1%	5.9%	6.2%
	Late resorptions *(percent of total implantations)*				
		0%	0%	0%	0.27%
	Live fetuses *(mean percent per litter)*				
		100%	100%	100%	100%
	Significant maternal mortality (16/51) occurred at 20 mg/kg-d. Percent resorptions and live fetuses based on number of surviving females at time of necropsy.				
Offspring growth					
Cholakis et al. (1980) Rabbits, NZW, 11–12/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 7–29	Doses	0	0.2	2.0	20
	Fetal body weight *(percent change compared to control)*				
		0%	−6.7%	−2.3%	−9.3%
Cholakis et al. (1980) Rats, F344, 24–25 females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants. 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 6–19	Doses	0	0.2	2.0	20
	Fetal body weight *(percent change compared to control)*				
		0%	2%	3%	−7%
	Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d.				

Reference and study design	Results				
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group) Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 2, 6, or 20 mg/kg-d Gavage GDs 6–15	Doses	0	2	6	20
	Fetal body weight *(percent change compared to control)*				
		0%	−4%	−2%	−9%[a]
	Fetal body length *(percent change compared to control)*				
		0%	−1%	−1%	−5%[a]
	Significant maternal mortality (16/51) occurred at 20 mg/kg-d.				
Morphological development					
Cholakis et al. (1980) Rabbits, NZW, 11–12/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 7–29	Doses	0	0.2	2.0	20
	Spina bifida *(incidence)*				
	Fetuses	0/88	0/99	0/94	3/110
	Litters	0/11	0/11	0/11	2/12
	Misshapen eye bulges *(incidence)*				
	Fetuses	0/88	0/99	0/94	3/110
	Litters	0/11	0/11	0/11	1/12
	Cleft palate *(incidence)*				
	Fetuses	0/39	1/46	2/44	2/52
	Litters	0/11	1/11	1/11	1/12
	Enlarged front fontanel *(incidence)*				
	Fetuses	0/49	5/53	2/50	8/58
	Litters	0/11	2/11	2/11	2/12
	Unossified sternebrae (*incidence*)				
	Fetuses	4/49	12/53	8/50	12/58
	Litters	4/11	7/11	4/11	6/12
Cholakis et al. (1980) Rats, F344, 24–25 females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants. 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 6–19	No gross or soft-tissue anomalies were seen in any exposure group. No treatment-related increase in the incidence of litters with skeletal anomalies was observed. Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d.				
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group) Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants	No treatment-related increase in the incidence of anomalies was observed.				
	Doses	0	2	6	20
	Total malformations *(percent of fetuses with malformations)*				
		1%	1%	0%	2%

Reference and study design	Results
0, 2, 6, or 20 mg/kg-d Gavage GDs 6−15	Significant maternal mortality (16/51) occurred at 20 mg/kg-d.

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Statistically significant dose-related trend ($p < 0.05$) by linear trend test, performed for this assessment. Average fetal weights or lengths for each litter comprised the sample data for this test.



Note: Filled circle indicates that response was statistically significantly different from the control.
(1) Statistically signficant dose-related trend (p <= 0.05) by linear trend test, performed for this assessment.

Figure 1-4. Exposure response array of developmental effects following oral exposure.

Integration of Developmental Effects

Developmental studies in rats (Angerhofer et al., 1986; Cholakis et al., 1980) demonstrated effects on offspring survival, growth, and morphological development only at doses associated with severe maternal toxicity and mortality. No dose-related developmental effects were observed in rabbits (Cholakis et al., 1980). As noted in EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), where adverse developmental effects are produced only at doses that cause minimal maternal toxicity, developmental effects should not be discounted as being secondary to maternal toxicity; however, at doses causing excessive toxicity, as is the case with RDX, information on developmental effects may be difficult to interpret and of limited value. At this time, the available data do not support developmental effects as a human hazard of RDX exposure.

1.2.5. Liver Effects

One occupational epidemiology study examined the association between RDX exposure and changes in serum liver enzymes. Case reports involving accidental exposure to RDX provide information on the potential for acute exposure to RDX to affect the liver in humans. In addition, organ weight, histopathology, and serum chemistry findings from experimental animal studies involving subchronic and chronic exposure to ingested RDX provide data relevant to an examination of the association between RDX exposure and liver effects. A summary of the liver effects associated with RDX exposure is presented in Tables 1-11 and 1-12 and Figure 1-5. Experimental animal studies are ordered in the evidence table and exposure-response array by duration of exposure and then by species.

Reports in humans provide inconsistent evidence of liver toxicity associated with acute exposure to RDX. Elevated serum levels of aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) were reported in several case reports of individuals who ingested unknown amounts of RDX (Küçükardali et al., 2003; Woody et al., 1986; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968) (see Appendix C, Section C.2). Liver biopsies did not reveal any abnormal observations (Stone et al., 1969). In other case reports, no significant changes in serum levels of liver enzymes were observed (Testud et al., 1996a; Ketel and Hughes, 1972). In a cross-sectional epidemiologic study of workers from five U.S. Army munitions plants (69 exposed to RDX alone and 24 to RDX and HMX; RDX exposure range: undetectable (<0.01 mg/m^3) to 1.6 mg/m^3) (Hathaway and Buck, 1977), serum chemistry analysis (including the serum liver enzymes AST, ALT, and alkaline phosphatase [ALP]) revealed no statistically significant differences between exposed and unexposed workers (see Table 1-11).

In experimental animals, some, but not all, subchronic studies reported increased liver weight associated with RDX exposure (see Table 1-12 and Figure 1-5). Dose-related increases in

relative liver weight[18] (11–25% in high-dose groups) were observed in male and female B6C3F1 mice given RDX in the diet for 90 days (Cholakis et al., 1980) and in female F344 rats in two separate 90-day dietary studies of RDX (Levine et al., 1990; Levine et al., 1981a, b; Cholakis et al., 1980); however, relative liver weights were not increased in female F344 rats in another 90-day gavage study (Crouse et al., 2006). Male F344 rats exhibited an increase in relative liver weight only in one of these subchronic studies (Levine et al., 1990; Levine et al., 1981a, b). In subchronic studies in other species, absolute liver weights were increased in male and female monkeys (6–16% relative to control at 1 and 10 mg/kg-day) (Martin and Hart, 1974) and in male, but not female, beagle dogs (53% relative to control in male dogs at 10 mg/kg-day) (Hart, 1974).

Chronic RDX exposures in B6C3F1 mice and F344 or Sprague-Dawley rats showed a less consistent pattern of liver weight increases. Interpretation of liver weight increases in the 2-year mouse study is complicated by the incidence of adenomas and carcinomas in each dose group; the apparent increase in liver weights in male and female mice exposed to RDX in diet (Lish et al., 1984) was reduced when mice with liver adenomas or carcinomas were removed from the analysis. In a 2-year rat study (Levine et al., 1983), relative liver weights were increased in high-dose (40 mg/kg-day) males and females (by 11 and 18% compared to controls, respectively), likely reflecting the depressed weight gain in the high-dose rats (2-30% in males and 10-15% in females). In evaluating organ weight data across studies of all durations, less emphasis is placed on evidence of organ weight changes from chronic (2-year) studies because normal physiological changes associated with aging and intercurrent disease contributes to inter-animal variability that could confound organ weight interpretation (Sellers et al., 2007), as is true of the mouse liver weight data for RDX.

Nonneoplastic histopathological changes in the liver were not associated with RDX exposure in the majority of experimental animal studies (Crouse et al., 2006; Levine et al., 1990; Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a, b; Hart, 1976, 1974; Martin and Hart, 1974), including 2-year oral studies in mice at doses up to 100 mg/kg-day (Lish et al., 1984) and in rats at doses up to 40 mg/kg-day (Levine et al., 1983). The few findings of liver lesions were reported in studies with more limited histopathological analyses, and were not confirmed in the studies with more complete histopathologic examination and longer exposure durations (Lish et al., 1984; Levine et al., 1983). For example, the incidence of liver portal inflammation was increased in female rats, but not male rats, exposed to 40 mg/kg-day in the diet for 90 days (Cholakis et al., 1980). There was an increase in the incidence of mild liver microgranulomas in female mice only (Cholakis et al., 1980) and karyomegaly of hepatocytes in male mice only exposed to

[18]Based on an evaluation of the relationship between organ weight and body/brain weight to determine which endpoint (organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio) is likely to more accurately detect target organ toxicity, Bailey et al. (2004) concluded that evaluation of the effects of a test chemical on liver weight are optimally analyzed using organ-to-body weight ratios. Therefore, the analysis of liver weight here focuses on relative weight data where study authors reported both relative and absolute weights, although both relative and absolute data are summarized in the evidence table (see Table 1-12).

320 mg/kg-day RDX in the diet for 90 days (Cholakis et al., 1980). Because both the rat and mouse studies by Cholakis et al. (1980) used relatively small group sizes (n = 10/sex/group) and provided histopathologic findings for the control and high-dose groups only, less emphasis is placed on these findings than on those from the 2-year bioassays. It should be noted that exposure to HMX, the primary contaminant in several of the RDX studies, was associated with histopathological changes in the livers of male rats fed doses ≥450 mg/kg-day for 13 weeks. However, similar findings were not observed in the RDX studies, where the doses of RDX employed in the studies would have resulted in HMX exposures of ≤60 mg/kg-day. The contribution of HMX exposure to the overall liver findings in the studies of RDX toxicity is therefore expected to be negligible.

Clinical chemistry parameters, including serum ALT, AST, and ALP, showed no treatment-related changes indicative of liver toxicity. Statistically significant changes in these parameters in some subchronic and chronic toxicity studies in rats and mice were relatively small (generally <50% of the control mean), were not dose-related in most instances, and showed no consistent pattern of change between sexes or across studies.

Some subchronic and chronic oral toxicity studies in rats and mice reported dose-related changes in serum cholesterol and triglyceride levels; however, these changes were not consistently observed in males and females within the same study, and patterns of changes were not consistent across studies. Specifically, serum triglyceride levels were elevated (up to 41%) in female B6C3F_1 mice exposed to RDX in the diet for 2 years, although increases were not dose-related (Lish et al., 1984); male mice in the same study did not show a similar increase in triglycerides. In contrast, serum triglycerides showed dose-related decreases in male and female F344 rats (50–62% at the high doses) in a subchronic oral (dietary) study (Levine et al., 1990; Levine et al., 1981a, b). In a chronic toxicity study by the same investigators (Levine et al., 1983), serum triglyceride levels were generally decreased in male and female rats (52 and 51%, respectively, at the highest dose of 40 mg/kg-day); however, triglyceride levels across the four dose groups in this study did not show a dose-related response.

Serum cholesterol levels showed a dose-related increase (38% at the high dose of 100 mg/kg-day) in female B6C3F_1 mice exposed to RDX in the diet for 2 years (Lish et al., 1984); however, changes in cholesterol in male mice in the same study were not dose related. Changes in serum cholesterol in male and female F344 rats exposed to RDX in the diet for 2 years at doses up to 40 mg/kg-day (Levine et al., 1983), in rats exposed to RDX by gavage for 90 days at doses up to 15 mg/kg-day (Crouse et al., 2006), and in monkeys exposed to RDX in the diet for 90 days (Martin and Hart, 1974) were relatively small (within 38% of control mean) and were not dose related.

Table 1-11. Evidence pertaining to liver effects in humans

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td rowspan="5">Hathaway and Buck (1977) (United States)
Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate).
Analysis group: limited to whites;
69 exposed to RDX alone and 24 exposed to RDX and HMX; 338 not exposed to RDX, HMX, or TNT.
Exposure measures: Exposure determination based on job title and industrial hygiene evaluation. Exposed subjects assigned to two groups: <LOD or ≥0.01 mg/m^3 (mean for employees with exposures ≥LOD: 0.28 mg/m^3).
Effect measures: Liver function tests.
Analysis: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and χ^2 tests for comparison of proportions).</td><td>Mean laboratory values of liver enzymes in men (mean; standard deviation not reported)
<table>
<tr><th rowspan="2">Test</th><th rowspan="2">Referent (n = 237)</th><th colspan="2">RDX exposed‡</th></tr>
<tr><th>Undetected (<LOD) (n = 22)</th><th>>0.01 mg/m^3 (n = 45)</th></tr>
<tr><td>LDH</td><td>173</td><td>191</td><td>174</td></tr>
<tr><td>ALP</td><td>82</td><td>78</td><td>80</td></tr>
<tr><td>ALA (SGOT)</td><td>22</td><td>25</td><td>21</td></tr>
<tr><td>AST (SGPT)</td><td>21</td><td>26</td><td>18</td></tr>
<tr><td>Bilirubin</td><td>0.5</td><td>0.4</td><td>0.4</td></tr>
</table>
‡Includes both workers exposed to RDX alone and RDX and HMX. No differences were statistically significant as reported by study authors. Similar results in women.</td></tr>
<tr><td>Liver function tests in men (prevalence of abnormally elevated values)
<table>
<tr><th rowspan="2">Test (abnormal range)</th><th rowspan="2">Referent</th><th colspan="2">RDX exposed‡</th></tr>
<tr><th>Undetected (<LOD)</th><th>>0.01 mg/m^3</th></tr>
<tr><td>LDH (>250)</td><td>2/237</td><td>1/22</td><td>0/45</td></tr>
<tr><td>ALP (>1.5)</td><td>34/237</td><td>1/22</td><td>6/45</td></tr>
<tr><td>AST (SGOT) (>35)</td><td>20/237</td><td>4/22</td><td>2/45</td></tr>
<tr><td>ALT (SGPT) (>35)</td><td>15/237</td><td>2/22</td><td>0/45</td></tr>
<tr><td>Bilirubin (>1.0)</td><td>5/237</td><td>1/22</td><td>1/45</td></tr>
</table></td></tr>
<tr><td>‡Includes both workers exposed to RDX alone and RDX and HMX. No differences were statistically significant as reported by study authors. Similar results in women.</td></tr>
</table>

LDH = lactate dehydrogenase; SGOT = glutamic oxaloacetic transaminase; SGPT = glutamic pyruvic transaminase

Table 1-12. Evidence pertaining to liver effects in animals

Reference and study design	Results					
Liver weight						
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100
	Absolute liver weight at 104 wks *(percent change compared to control)*					
	M	0%	28%*	11%	12%	35%*
	F	0%	7%	7%	15%	18%*
	Relative liver weight at 104 wks *(percent change compared to control)*					
	M	0%	32%*	12%	14%	46%*
	F	0%	6%	8%	18%	45%*
	Note: Percent change in liver weights of male and female mice was reduced in all dose groups when mice with liver tumors were removed from the analysis, suggesting no real effect on liver weight.					
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Doses	0	1.0	3.1	10	
	Absolute liver weight *(percent change compared to control)*					
	M	0%	−6%	−6%	−6%	
	F	0%	7%	−11%	1%	
	Relative liver weight *(percent change compared to control)*					
	M	0%	−5%	−2%	−3%	
	F	0%	17%	−2%	13%	
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Absolute liver weight at 105 wks *(percent change compared to control)*					
	M	0%	3%	−7%	1%	−8%
	F	0%	1%	−4%	3%	0%
	Relative liver weight at 105 wks *(percent change compared to control)*					
	M	0%	1%	0%	2%	11%
	F	0%	1%	−2%	6%	18%*
Cholakis et al. (1980) Mice, B6C3F$_1$, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute liver weight *(percent change compared to control)*						
	M	0%	–	–	–	−6%	−5%
	F	0%	–	–	–	−4%	−1%
	Relative liver weight *(percent change compared to control)*						
	M	0%	–	–	–	−4%	−4%
	F	0%	–	–	–	−6%	1%

Reference and study design	Results						
Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Doses	0		80	160		320
	Absolute liver weight *(percent change compared to control)*						
	M	0%		2%	12%		26%*
	F	0%		4%	9%		29%*
	Relative liver weight *(percent change compared to control)*						
	M	0%		0%	9%		25%*
	F	0%		4%	4%		22%*
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute liver weight *(percent change compared to control)*						
	M	0%	–	–	–	−2%	−5%
	F	0%	–	–	–	6%	4%
	Relative liver weight *(percent change compared to control)*						
	M	0%	–	–	–	2%	3%
	F	0%	–	–	–	10%	11%
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	Doses	0		5	16		50
	Absolute liver weight *(percent change compared to control)*						
	M	0%		7%	−16%		–
	F	0%		0%	−14%		–
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Absolute liver weight *(percent change compared to control)*						
	M	0%	−6%	−9%	0%	7%	5%
	F	0%	1%	7%	18%*	15%	28%*
	Relative liver weight *(percent change compared to control)*						
	M	0%	0%	−1%	2%	5%	2%
	F	0%	1%	−2%	2%	−3%	2%

Reference and study design	Results						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 3–4 wks old; 10/sex/group; 30/sex/group for controls 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Doses	0	10	30	100	300	600
	Absolute liver weight *(percent change compared to control)*						
	M	0%	5%	−1%	−2%	–	–
	F	0%	2%	4%	16%*	–	–
	Relative liver weight *(percent change compared to control)*						
	M	0%	9%	6%	20%	–	–
	F	0%	3%	5%	19%*	–	–
Hart (1974)[c] Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0	0.1	1	10		
	Absolute liver weight *(percent change compared to control)*						
	M	0%	–	–	53%		
	F	0%	–	–	3%		
Martin and Hart (1974)[c] Monkeys, Cynomolgus or Rhesus[d], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10		
	Absolute liver weight *(percent change compared to control)*						
	M + F	0%	2%	6%	16%		
Histopathological lesions							
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Histopathological lesions in liver other than adenomas and carcinomas were not significantly different compared to controls, as reported by study authors.						
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Histopathological examination performed only for controls and 10 mg/kg-d rats; no significant differences compared to controls were reported by study authors.						

Reference and study design	Results					
Levine et al. (1983) Rats, F344, 3–4 wks old; 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Microgranulomas *(incidence)*					
	M	0/38	0/36	0/25	0/29	0/4
	F	10/43	19/45	12/42	17/41	4/28
Cholakis et al. (1980) Mice, B6C3F$_1$, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 80, 60, or 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wks	Doses	0	80	160	320	
	Liver microgranulomas; mild *(incidence)*					
	M	2/10	–	–	1/9	
	F	2/11	–	–	7/11*	
	Increased karyomegaly of hepatocytes *(incidence)*					
	M	0/10	–	–	5/9*	
	F	–	–	–	–	
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Liver granulomas; mild *(incidence)*						
	M	0/10	–	–	–	–	1/10
	F	–	–	–	–	–	–
	Liver portal inflammation *(incidence)*						
	M	2/10	–	–	–	–	3/10
	F	1/10	–	–	–	–	7/10
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Histopathology examination of the 15 mg/kg-d group showed one male with mild liver congestion and one female with a moderate-sized focus of basophilic cytoplasmic alteration; neither finding was attributed by study authors to RDX treatment.						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Histopathological examination of liver did not reveal any significant differences compared to controls, as reported by study authors. No histopathology findings available for the 300 or 600 mg/kg-d dose groups because all rats in these groups died before the 13-wk necropsy.						

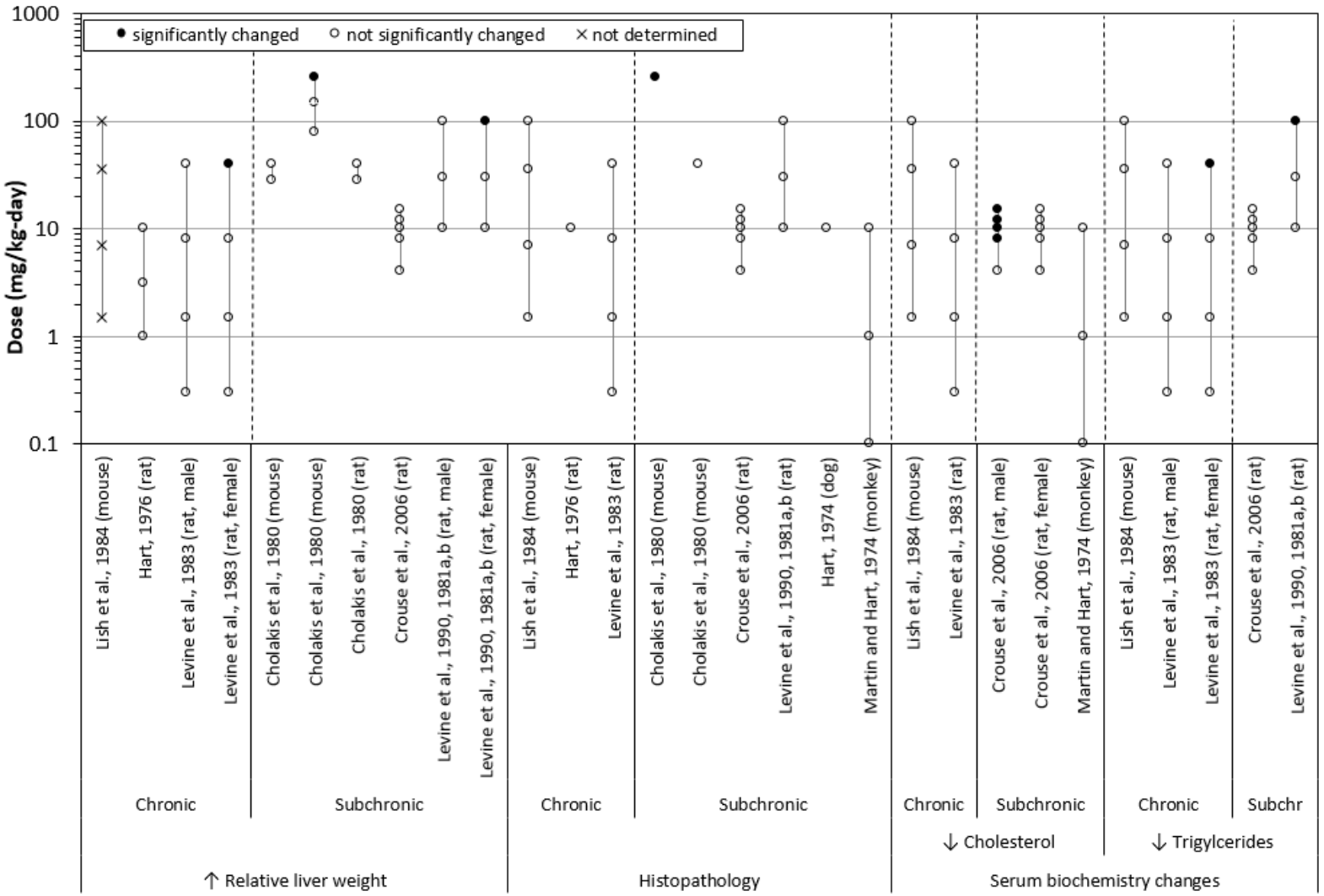
Reference and study design	Results					
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Histopathological examination performed only for controls and 10 mg/kg-d dogs; no significant differences compared to controls were reported.					
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus[d], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	An increase in the amount of iron-positive material in liver cord cytoplasm was reported in monkeys treated with 10 mg/kg-d RDX, which the study authors considered to be of uncertain toxicological significance. Because iron-positive stain was present in controls and no further characterization of the staining was provided in the study report, the toxicological significance of this finding could not be determined.					
Serum chemistry						
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100
	Serum cholesterol at 105 wks *(percent change compared to control)*					
	M	0%	11%	−11%	5%	39%
	F	0%	5%	15%	25%	38%
	Serum triglycerides at 105 wks *(percent change compared to control)*					
	M	0%	21%	−20%	10%	−25%
	F	0%	34%	28%	41%	28%
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Serum cholesterol at 104 wks *(percent change compared to control)*					
	M	0%	15%	38%	19%	−6%
	F	0%	6%	3%	−7%	−9%
	Serum triglycerides at 104 wks *(percent change compared to control)*					
	M	0%	14%	−15%	−12%	−52%
	F	0%	18%	5%	−42%	−51%*

Reference and study design	Results						
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Serum cholesterol *(percent change compared to control)*						
	M	0%	−3%	−10%*	−16%*	−18%*	−11%*
	F	0%	−1%	−8%	−4%	−4%	−1%
	Serum triglycerides *(percent change compared to control)*						
	M	0%	1%	1%	−7%	−2%	−19%
	F	0%	−16%	−21%	7%	−37%	18%
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Doses	0	10	30	100	300	600
	Serum triglyceride levels *(percent change compared to control)*						
	M	0%	−14%	−34%	−62%*	–	–
	F	0%	−12%	−29%	−50%*	–	–
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus[d], 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Serum biochemistry analysis revealed scattered deviations, but study authors indicated they appear to have no toxicological significance.						
	Doses	0		0.1	1		10
	Serum cholesterol *(percent change compared to control)*						
	M	0%		−17%	−2%		−7%
	F	0%		7%	7%		7%

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[c]Liver weight data from the Hart (1974) and Martin and Hart (1974) studies were considered less informative than other studies. Hart (1974) reported organ weight data for high-dose dogs (3/sex/group) only, and the liver weights from Martin and Hart (1974) were highly variable across monkeys (e.g., liver weights for the control animals ranged from 46 to 141 g). Therefore, liver weight data from these two studies were not presented in the exposure-response array for liver effects (Figure 1-5).
[d]The species of monkey used in this study was inconsistently reported in the study as either Cynomolgus (in the methods section) or Rhesus (in the summary).

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

1



Note: Filled circle indicates that response was statistically significantly different from the control.
X - Not considered due to confounding caused by presence of tumors.

Figure 1-5. Exposure response array of liver effects following oral exposure.

Integration of Liver Effects

There is limited evidence from human studies and from studies in experimental animals that RDX may affect the liver. The observation of transient elevations of serum liver enzymes in several human case reports of individuals who ingested unknown amounts of RDX suggests that RDX might target the liver; however, serum liver enzymes were not elevated in a small cross-sectional study of munition plant workers exposed to RDX. In experimental animals, dose-related increases in liver weight were observed in some studies following subchronic oral exposure, but liver weight changes were not consistent across sexes within a study or across different studies. Changes in serum chemistry were not consistent across studies and the magnitude of change relative to concurrent controls was not indicative of liver damage. Nonneoplastic histopathologic lesions of the liver were also not consistently associated with RDX exposure. At this time, the available data do not support liver effects as a human hazard of RDX exposure.

1.2.6. Other Noncancer Effects

There are some reports that RDX may induce effects on the eyes, on the cardiovascular, musculoskeletal, immune, gastrointestinal (GI), hematological, and male reproductive systems, and on body weight. However, there is less evidence for these effects compared to organ systems described earlier in Section 1.2. Generally, human evidence for effects in these organ systems is limited to human case reports. Evidence of effects in experimental animals is generally inconsistent across studies of similar duration in the same species, or lacks consistent, dose-related patterns of increasing or decreasing effect. A summary of the evidence for an association between these other noncancer effects and RDX exposure is provided below; a more detailed discussion is provided in Appendix C.3.2. As discussed below, the information to assess the association between RDX exposure and toxicity for the organ systems presented below is considered inadequate.

Ocular Effects

There is no human evidence of ocular effects following exposure to RDX. In animals, the incidence of cataracts was significantly increased in high-dose female rats (73%) relative to controls (32%) in one chronic oral study (Levine et al., 1983). This finding was not observed in males in the same chronic study or in other chronic or subchronic studies in rats, mice, or monkeys (Crouse et al., 2006; Lish et al., 1984; Cholakis et al., 1980; Martin and Hart, 1974). There is insufficient information to assess ocular toxicity following exposure to RDX.

Cardiovascular Effects

Human evidence of cardiovascular effects consists of case reports of transient arterial hypertension, sinus tachycardia, and premature ventricular beats in male workers or men who accidentally ingested RDX (Küçükardali et al., 2003; Barsotti and Crotti, 1949). In animals, evidence is limited to inconsistent findings of changes in heart weight and a report of increased incidence of minimal histopathological changes in a 90-day rat study at a dose that also produced 40% mortality

(Cholakis et al., 1980). There is insufficient information to assess cardiovascular effects following exposure to RDX.

Musculoskeletal Effects

Evidence for musculoskeletal effects in humans is limited to case reports that described muscle twitches, soreness, and muscle injury as indicated by elevated levels of aspartate aminotransferase (AST), creatine phosphokinase, and myoglobinuria (Testud et al., 2006; Küçükardali et al., 2003; Hett and Fichtner, 2002; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968). In animal studies, evaluations of muscle and skeletal tissues did not reveal any histopathological alterations in rats or mice following chronic exposure or in mice, rats, or dogs following subchronic exposure. There is insufficient information to assess musculoskeletal effects following exposure to RDX.

Immune System Effects

Increased white blood cell (WBC) counts were reported in several case reports of humans acutely exposed to RDX (Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968). In animals, there were no consistent patterns of change in WBC count or spleen weight across the RDX database. No dose-related immune effects were observed in a 90-day study in F344 rats that evaluated structural measures of immunotoxicity (including red blood cell [RBC] and WBC populations, proportion of cell surface markers, cellularity in proportion to organ weight, B and T cells in the spleen, and CD4/CD8 antigens of maturing lymphocytes in the thymus) (Crouse et al., 2006). None of the available studies included evaluation of more sensitive measures of functional immune system changes. Therefore, there is insufficient information to assess immunotoxicity following exposure to RDX.

Gastrointestinal Effects

Nausea, vomiting, and erosive gastroduodenitis were identified in human case reports of RDX poisonings, generally concurrent with severe neurotoxicity (Kasuske et al., 2009; Davies et al., 2007; Küçükardali et al., 2003; Hett and Fichtner, 2002; Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968; Kaplan et al., 1965; Barsotti and Crotti, 1949). There have been similar reports of vomiting in swine, dogs, and monkeys (Musick et al., 2010; Hart, 1974; Martin and Hart, 1974). Generally, histopathological changes of the GI tract were not observed in RDX-exposed animals. There is insufficient information to assess gastrointestinal toxicity following exposure to RDX.

Hematological Effects

Temporary hematological alterations, including anemia, decreased hematocrit, hematuria, and methemoglobinemia were observed in some human case reports following acute exposure (Kasuske et al., 2009; Küçükardali et al., 2003; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968). Observations of anemia in case reports may reflect co-exposure to 2,4,6-trinitrotoluene (TNT). Levine and colleagues identified that anemia resulted from exposure to TNT in F344 rats, but not RDX (Levine et al., 1990; Levine et al., 1981a, b). Hematological findings in a case-control and cross-sectional occupational study were inconsistent (West and Stafford, 1997; Hathaway and Buck, 1977); both studies used small sample sizes and were considered low confidence studies. In general, subchronic and chronic animal studies showed no consistent dose-related patterns of change in hematological parameters. There is insufficient information to assess hematological toxicity following exposure to RDX.

Reproductive Effects

Investigation of the potential effects of RDX on reproductive function is limited to a two-generation study in rats by Cholakis et al. (1980) that also included a dominant lethal mutation study. A reduction in number of pregnancies was reported only at a dose that also resulted in decreased food consumption, decreased body weight gain, and increased mortality. The limited investigation of reproductive function in RDX-exposed rats by a single investigator provides insufficient information to assess female reproductive toxicity following exposure to RDX.

Evidence of male reproductive toxicity comes largely from the finding of increased incidence of testicular degeneration in male B6C3F$_1$ mice exposed to ≥35 mg/kg-day RDX for 2 years in the diet compared to concurrent controls (Lish et al., 1984). The biological significance of this finding is unclear because no similar histopathological changes were observed in this study at 6 or 12 months, durations longer than the 1.4-month duration of spermatogenesis in mice, and because of the loss of testicular function that occurs in aging rodents. The evidence for testicular degeneration in mice suggested by Lish et al. (1984) was generally not supported by histopathological findings in male reproductive organs in other studies, and changes in testes weight across the RDX database were generally small, not dose-related, and directionally inconsistent. There is insufficient information to assess male reproductive toxicity following exposure to RDX.

Body Weight Effects

Changes in body weight gain were reported in experimental animal studies involving chronic and subchronic exposure to ingested RDX, but generally at high doses that were also associated with elevated mortality or with severe kidney and urinary bladder toxicity in male rats in the case of the Levine et al. (1983) study. For the most part, at lower doses, there were no apparent patterns of treatment-related body weight changes across dose groups or sexes within a study or across studies. Thus, available studies of RDX provide evidence that RDX exposure causes

decreases in body weight gain in mice and rats, but these effects appear to be secondary to effects on other primary targets of RDX toxicity.

1.2.7. Carcinogenicity

The relationship between exposure to RDX and cancer has not been investigated in human populations. The carcinogenicity of RDX has been examined in one oral chronic/carcinogenicity bioassay in mice (Lish et al., 1984) and two bioassays in rats (Levine et al., 1983; Hart, 1976). The 2-year studies by Lish et al. (1984) and Levine et al. (1983) included comprehensive histopathological examination of major organs, multiple dose groups and a control, and >50 animals/dose group (plus additional interim sacrifice groups). In both studies, the maximum tolerated dose was reached or exceeded in high-dose animals (based on decreased terminal body weight in high-dose male and female mice of 5 and 19%, respectively, and decreased survival in male and female rats by approximately 50 and 25%, respectively, compared to the control).[19] The earlier Hart (1976) study is largely limited by the lack of characterization of the test material and histopathologic examination in control and high-dose groups only. A temperature spike in the animal rooms on study day 76 resulted in significant mortality across all dose groups and control animals; however, there were still >80 rats/sex/group after the overheating incident and ≥50 rats/sex/group at termination, and it seems unlikely that the mortality associated with the temperature spike would have affected a tumor response in the rats. A peer review of histopathological evaluations by the study pathologist was performed only for female mouse liver tissues from the Lish et al. (1984) study (see discussion of the Pathology Working Group (PWG) below). A summary of the evidence for liver and lung tumors in experimental animals from these three bioassays is provided in Tables 1-13 and 1-14.

Liver Tumors

An increased incidence of liver tumors was observed in one chronic mouse study (Lish et al., 1984) and one of two chronic rat studies (Levine et al., 1983). Incidences of hepatocellular tumors are presented in Table 1-13 and discussed in further detail below.

The incidence of hepatocellular carcinomas and the combined incidence of hepatocellular adenomas or carcinomas showed a statistically significant positive trend with RDX dose in female, but not male, B6C3F_1 mice as compared to concurrent controls in a 2-year dietary study (Lish et al., 1984). In female B6C3F_1 mice, Lish et al. (1984) observed that the liver tumor incidence in the concurrent female control mice was relatively low (1/65), and significantly lower than the

[19]In high-dose mice in the Lish et al. (1984) study, reduced survival due to acute RDX toxicity occurred during the first 11 weeks on study at a dietary dose of 175 mg/kg-day; survival in high-dose animals was similar to controls after 11 weeks when the dose was reduced to 100 mg/kg-day. By contrast, in high-dose rats in the Levine et al. (1983) study, elevated mortality, particularly in males, occurred gradually over the entire period of the study beginning after 6 months, and was attributable in large part to kidney toxicity.

incidence from historical controls (historical incidence data not provided by study authors). The study authors also compared liver tumor incidence in RDX-exposed female mice to mean historical control incidence for female mice of the same strain from National Toxicology Program (NTP) studies conducted during the same time period (147/1,781 or 8%; range: 0–20%) (Haseman et al., 1985).[20] The combined incidence of hepatocellular adenomas or carcinomas in female mice at RDX doses ≥35 mg/kg-day (19% at both doses) was statistically significantly elevated when statistical analysis was performed using NTP historical control data; limitations associated with comparisons to historical control data originating from a different laboratory are acknowledged given cross-study differences in diet, laboratory, pathological evaluation, and animal provider.

A PWG reviewed the slides of female mouse liver lesions from the Lish et al. (1984) study (Parker et al., 2006; Parker, 2001). Some malignant tumors were downgraded to benign status, and several lesions initially characterized as adenomas were changed to non-neoplastic lesions based on more recent diagnostic criteria used by the PWG (Harada et al., 1999). There remained a statistically significant positive trend in the combined incidence of hepatocellular adenomas or carcinomas, consistent with the original findings of Lish et al. (1984). Because the PWG analysis reflects more recent histopathological criteria for the grading of tumors, the incidence of hepatocellular adenomas or carcinomas as reported by Parker et al. (2006) were considered the more appropriate measure of liver tumor response in female mice from the Lish et al. (1984) bioassay. The PWG also offered observations about the histopathology methods used by Lish et al. (1984) that raised some concerns about the uniformity of histologic processing (Parker et al., 2006; Parker, 2001). These included variation in size and shape of sections, suggesting that liver sections were not uniformly taken from the same area of the liver of all animals; only one liver section present from most animals (two sections are commonly examined in current carcinogenicity bioassays); and more than one section prepared for 20 mice across different groups, raising some concern of sample bias but likely reflecting sections taken from visible lesions at gross necropsy.

In male mice from the Lish et al. (1984) study, the incidences of hepatocellular carcinomas in treated groups were higher than in the control, and the combined incidences of hepatocellular adenomas or carcinomas of male mice were higher in three of four treated groups than in the control; however, there were no statistically significant trends in either case. The incidences of liver carcinoma in control (21%) and treated groups of male mice (22–33%) were generally within

[20]Comparison of control incidences of hepatocellular adenomas or carcinomas between Lish et al. (1984) and Haseman et al. (1985) must be interpreted with caution because of cross-study differences in labs, diets, and sources of animals. Specifically, the labs used by NTP and analyzed by Haseman et al. (1985) did not include the lab contracted to perform the Lish et al. (1984) study, and it is not clear if the diet used in the Lish et al. (1984) study was included in the diets reported in the NTP studies. Further, the NTP studies included three different suppliers of mice; one supplier was also used in the Lish et al. (1984) study. EPA *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005a) also note that, unless the tumor is rare, the standard for determining statistical significance of tumor incidence is a comparison of dosed animals with the concurrent controls.

the range for the same mouse strain reported by NTP (8–32%) (Haseman et al., 1985). Similarly, the combined incidences of liver adenoma or carcinoma in control (32%) and treated groups (27–48%) were within the range for the same mouse strain reported by NTP (14–58%) (Haseman et al., 1985).[21] The PWG did not re-analyze liver tumor slides from male mice; the SAB (SAB, 2017) noted this as unusual since typically sections from both male and female animals are reevaluated to ensure that findings in both sexes are reliable.

In the two-year bioassay in F344 rats (Levine et al., 1983), RDX was not associated with dose-related increases in the incidence of nonmalignant liver tumors (neoplastic nodules) or combined incidence of liver neoplastic nodules or carcinomas.[22] However, a statistically significant positive trend with dose was observed in the incidence of hepatocellular carcinomas in male, but not female, F344 rats (Levine et al., 1983). In the Levine et al. (1983) study, there were only a few tumors observed in the exposed groups of male rats (0/55, 0/52, 2/55, 2/31) relative to the control (1/55), and inferences made from such a sparse response are uncertain. Because hepatocellular carcinomas are rare tumors in the rat[23], some perspective is obtained by considering historical control data. In a paper published concurrently with the Levine et al. (1983) study, NTP reported an incidence of liver carcinomas in untreated control male F344 rats of 0.7% (12/1,719; range: 0–2%) (Haseman et al., 1985). In Levine et al. (1983), the incidence of liver carcinomas in control male rats (1/55 or 1.8%) was at the upper end of this NTP range, and the incidence in RDX-treated male F344 rats in the highest two dose groups (3.6 and 6.4%) exceeded the NTP historical control range. Using incidence data from NTP historical controls, the trend for carcinoma in the RDX-treated F344 rats was statistically significant (p-value = 0.003; one-sided exact Cochrane-Armitage trend test). It should be noted that although the NTP historical controls (Haseman et al., 1985) are comparable with Levine et al. (1983) in terms of the time period, they may not be directly comparable in terms of diet, laboratory, pathological evaluation, and animal provider. However, other historical control datasets from male F344 rats, both recent and of the time period of the Levine study, indicate similar low incidences of liver carcinomas (0.36%, (NTP, 2009); 0.31%, (Maita et al., 1987)). In the Levine et al. (1983) study, mortality in the highest dose group was substantially higher than in the other dose groups during the second year leading to uncertainty in the true cancer incidence in the high dose group. It was not possible to estimate mortality-adjusted incidences because no time-to-death information was available.

In a second 2-year dietary study using a different rat strain (Sprague-Dawley), the combined incidence of hepatocellular adenomas or carcinomas was not increased with dose in rats

[21]Considerations listed in footnote 20 apply to the comparison of combined liver adenoma and carcinoma incidence to historical controls as well.

[22]The incidence of neoplastic nodules of 7.3% in control male rats in Levine et al. (1983) was consistent with the NTP historical control range of 0–12% (mean: 3.5% or 61/1,719) (Haseman et al., 1985).

[23]NTP historical control data for hepatocellular carcinomas F344 rats as reported in Haseman et al. (1985): 12/1,719 (0.7%) in males; 3/1,766 (0.17%) in females. Historical control data for Charles River Sprague-Dawley rats as reported in Chandra et al. (1992): 6/1,340 (0.45%) in males; 1/1,329 (0.08%) in females.

of either sex at doses up to 10 mg/kg-day (Hart, 1976). However, interpretation of results from this study is limited by the comparatively lower doses employed in the study, and the recording of effects only at the control and high dose groups.

Table 1-13. Liver tumors observed in chronic animal bioassays

Reference and study design	Results[a]					
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100[b]
	Hepatocellular adenomas *(incidence)*[c]					
	M	8/63 (12.7)	6/60 (10.0)	1/62* (1.6)	7/59 (11.9)	7/27 (25.9)
	F	1/65 (1.5)	1/62 (1.6)	6/64 (9.4)	6/64 (9.4)	3/31 (9.7)
	Hepatocellular carcinomas *(incidence)*[c]					
	M	13/63 (20.6)	20/60 (33.3)	16/62 (25.8)	18/59 (30.5)	6/27 (22.2)
	F	0/65 (0.0)	4/62 (6.5)	3/64 (4.7)	6/64 (9.4)	3/31[d] (9.7)
	Hepatocellular adenoma or carcinoma combined *(incidence)*[c]					
	M	20/63 (31.7)	26/60 (43.3)	17/62 (27.4)	25/59 (42.4)	13/27 (48.1)
	F	1/65 (1.5)	5/62 (8.1)	9/64* (14.1)	12/64* (18.8)	6/31*[d] (19.4)
	PWG reanalysis of liver lesion slides from female mice (Parker et al., 2006; Parker, 2001).[e]					
	Doses	0	1.5	7.0	35	175/100[b]
	Hepatocellular adenomas *(incidence)*[c]					
	F	1/67 (1.5)	3/62 (4.8)	2/63 (3.2)	8/64 (12.5)	2/31 (6.5)
	Hepatocellular carcinomas *(incidence)*[c]					
	F	0/67 (0.0)	1/62 (1.6)	3/63 (4.8)	2/64 (3.1)	2/31 (6.5)
	Hepatocellular adenoma or carcinoma combined *(incidence)*[c]					
	F	1/67 (1.5)	4/62 (6.5)	5/63 (7.9)	10/64 (15.6)	4/31[d] (12.9)
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	**Doses**	0	1.0	3.1	10	
	Neoplastic nodules *(incidence)*[c]					
	M	0/82	–	–	3/77	
	F	1/72	–	–	1/81	
	Hepatocellular carcinomas *(incidence)*[c]					

Reference and study design	Results[a]				
	M	1/82	–	–	1/77
	F	1/72	–	–	1/81[f]
	Neoplastic nodules or hepatocellular carcinomas combined *(incidence)*[c]				
	M	1/82	–	–	4/77
	F	2/72	–	–	2/81
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Neoplastic nodules *(incidence)*[c]					
	M	4/55 (7.3)	3/55 (5.5)	0/52 (0.0)	2/55 (3.6)	1/31 (3.2)
	F	3/53 (5.6)	1/55 (1.8)	1/54 (1.9)	0/55 (0.0)	4/48 (8.3)
	Hepatocellular carcinomas *(incidence)*[c]					
	M	1/55 (1.8)	0/55 (0.0)	0/52 (0.0)	2/55 (3.6)	2/31[d] (6.5)
	F	0/53 (0.0)	1/55 (1.8)	0/54 (0.0)	0/55 (0.0)	0/48 (0.0)
	Neoplastic nodules or hepatocellular carcinomas combined *(incidence)*[c]					
	M	5/55 (9.1)	3/55 (5.5)	0/52 (0.0)	4/55 (7.3)	3/31 (9.7)
	F	3/53 (5.6)	2/55 (3.6)	1/54 (1.9)	0/55 (0.0)	4/48 (8.3)

*Statistically significant difference compared to the control group ($p < 0.05$), identified by the authors.

[a]Selected percent incidences are provided in parentheses below the incidences to help illustrate patterns in the responses.

[b]The lower dose of 100 mg/kg-day was started in week 11, resulting in a duration-weighted average dose of 107 mg/kg-day.

[c]The incidences reflect the animals surviving to month 12.

[d]Statistically significant trend ($p < 0.05$) was identified using a one-sided Cochran-Armitage trend tests performed by EPA.

[e]The numbers of animals at risk (i.e., the denominators) in the control group (n = 67) and 7 mg/kg-day dose group (n = 63) as reported in the PWG reanalysis (Parker et al., 2006; Parker, 2001) differed from the numbers reported in the original study by Lish et al. (1984) (n = 65 and 64, respectively). Further investigation of these differences by the U.S. Army (sponsor of the mouse bioassay and subsequent PWG reevaluation) was unable to resolve the discrepancy (email to Louis D'Amico, U.S. EPA, from Mark Johnson, U.S. Army Public Health Command, February 13, 2015).

[f]Hart (1976) distinguishes the single high-dose carcinoma in the liver from a hepatocellular carcinoma; the incidence of hepatocellular carcinomas in this dose group is shown as 0/81 (p. 119 of the publication).

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

Lung Tumors

Lung tumors were observed in female and male B6C3F$_1$ mice exposed to RDX in the diet for 2 years (Lish et al., 1984) (see Table 1-14). Incidence of alveolar/bronchiolar carcinomas and the combined incidence of alveolar/bronchiolar adenomas or carcinomas showed a statistically significant positive trend (one-sided *p*-values of 0.016 and 0.009, respectively, for the Cochran-Armitage trend test) in female mice. Incidence of alveolar/bronchiolar carcinomas in male mice showed a statistically significant positive trend (*p*-value = 0.015; one-sided Cochran-Armitage trend test). However, the combined incidence of adenomas and carcinomas was not elevated in male mice. In such a case, NTP policy recommends analyzing the tumors both separately and in combination (McConnell et al., 1986). This recommendation arose out of concern that combining benign and malignant neoplasms can result in a false negative if the chemical shows a statistically significant increase in malignant tumors without an increase in the combined incidence. In an addendum to the study report that included results of additional examination and sectioning of lung specimens from the mid-dose groups in the mouse study, Lish et al. (1984) noted an increase in the combined incidences of primary pulmonary neoplasms in males of all dose groups and in females in the 7.0, 35, and 175/100 mg/kg-day dose groups, but regarded these neoplasms as random and not biologically significant (rationale for this conclusion not provided).

Bioassays in rats provide no evidence of an association between RDX exposure and induction of lung tumors. The incidence of alveolar/bronchiolar adenomas or carcinomas was not increased in either sex of Sprague-Dawley rats exposed chronically to RDX at doses up to 10 mg/kg-day (Hart, 1976) or in F344 rats of either sex exposed chronically to RDX at doses up to 40 mg/kg-day (Levine et al., 1983). Alveolar/bronchiolar carcinomas are rare tumors in both species of rats, male or female (Chandra et al., 1992; Haseman et al., 1985).

Table 1-14. Lung tumors observed in chronic animal bioassays

Reference and study design	Results[a]					
Lish et al. (1984) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100[b]
	Alveolar/bronchiolar adenomas *(incidence)*[c]					
	M	6/63 (9.5)	5/60 (8.3)	5/62 (8.1)	7/59 (11.9)	1/27 (3.7)
	F	4/65 (6.2)	2/62 (3.2)	5/64 (7.8)	9/64 (14.1)	3/31 (9.7)
	Alveolar/bronchiolar carcinomas *(incidence)*[c]					
	M	3/63 (4.8)	6/60 (10.0)	3/62 (4.8)	7/59 (11.9)	5/27[d] (18.5)
	F	3/65 (4.6)	1/62 (1.6)	3/64 (4.7)	3/64 (4.7)	4/31[d] (12.9)
	Alveolar/bronchiolar adenoma or carcinoma combined *(incidence)*[c]					

Reference and study design	Results[a]					
	M	9/63 (14.3)	11/60 (18.3)	8/62 (12.9)	14/59 (23.7)	6/27 (22.2)
	F	7/65 (10.8)	3/62 (4.8)	8/64 (12.5)	12/64 (18.8)	7/31[d] (22.6)
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Doses	0	1.0	3.1		10
	Alveolar/bronchiolar adenoma *(incidence)*					
	M	2/83	–	–		1/77
	F	0/73	–	–		0/82
	No alveolar/bronchiolar carcinomas reported by study authors.					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Alveolar/bronchiolar adenomas *(incidence)*[c]					
	M	1/55	0/15	1/17	0/16	1/31
	F	3/53	0/7	0/8	1/10	0/48
	Alveolar/bronchiolar carcinomas *(incidence)*[c]					
	M	–	–	–	–	–
	F	0/53	0/7	1/8	0/10	0/48
	Alveolar/bronchiolar adenoma or carcinoma combined *(incidence)*[c]					
	M	–	–	–	–	–
	F	3/53	0/7	1/8	1/10	0/48

[a]Selected percent incidences are provided in parentheses below the incidences to help illustrate patterns in the responses.
[b]The lower dose of 100 mg/kg-day was started in week 11, resulting in a duration-weighted average dose of 107 mg/kg-day.
[c]The incidences reflect the animals surviving to month 12.
[d]Statistically significant trend ($p < 0.05$) was identified using a one-sided Cochran-Armitage trend test performed by EPA.

Note: A dash ("–") indicates that the study authors did not measure or report a value for that dose group.

Mechanistic Evidence

There are few mechanistic data to inform a MOA determination for either liver or lung tumors induced by exposure to RDX.

Largely negative findings in in vitro and in vivo genotoxicity assay for parent RDX or its oxidative metabolites (see Appendix C, Section C.3.2) suggest that parent RDX or its oxidative metabolites do not interact directly with deoxyribonucleic acid (DNA). In contrast, there are some positive genotoxicity results for the N-nitroso metabolites of RDX, specifically hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). Trace

amounts of MNX and TNX metabolites were identified in minipigs orally exposed to ^{14}C-RDX in an ADME study; minipigs were chosen as the animal model for investigation of RDX metabolism because the GI tract of pigs more closely resembles that of humans (Musick et al., 2010; Major et al., 2007). MNX has tested positive in some in vitro assays, including unscheduled DNA synthesis in primary rat hepatocytes and the mouse lymphoma forward mutation assay (Snodgrass, 1984), although MNX tested negative in the only in vivo test performed, a mouse dominant lethal mutation test (Snodgrass, 1984). MNX was not mutagenic in *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), with or without the addition of the S9 metabolic activating mixture (Pan et al., 2007b; Snodgrass, 1984). When *S. typhimurium* strains TA97a and TA102, strains sensitive to frame shift and oxidative DNA damage, were used in conjunction with elevated concentrations of the metabolizing system (S9), MNX and TNX were mutagenic. N-nitroso metabolites, including MNX and TNX, are generated anaerobically and are likely a result of bacterial transformation of parent RDX in the GI tract to various N-nitroso derivatives (Pan et al., 2007b). Exposure to potentially mutagenic N-nitroso metabolites of RDX generated in the GI tract of mice may occur in the liver (and subsequently in the systemic circulation) via enterohepatic circulation. However, as noted earlier in pigs, the N-nitroso metabolites of RDX have been identified only in trace amounts in urine compared to the major metabolites, 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diaza-butanamide (Major et al., 2007). Thus, the contribution of the N-nitroso metabolites to the overall carcinogenic potential of RDX is unclear.

Aberrant expression of miRNAs was observed in the brains and livers of female B6C3F$_1$ mice fed 5 mg RDX/kg in the diet for 28 days (Zhang and Pan, 2009b) (dose of 0.75–1.5 mg/kg-day estimated by Bannon et al. (2009b)), with several oncogenic miRNAs being upregulated, while several tumor-suppressing miRNAs were downregulated. However, the pattern of induction was not always consistent in the livers of RDX-treated mice (e.g., miR-92a was downregulated in liver tissue samples when it is typically upregulated in hepatocellular carcinomas) (Sweeney et al., 2012b). miRNAs have been associated with several cancers (Wiemer, 2007; Zhang et al., 2007), but the utility of miRNAs as predictive of carcinogenesis has not been demonstrated (Bannon et al., 2009b). Further, it is unknown whether or not aberrant expression of a specific miRNA (or suite of miRNAs) plays a role in the MOA of RDX carcinogenicity. Microarray analysis of gene expression in male Sprague-Dawley rats after exposure to a single oral (capsule) dose of RDX revealed a general upregulation in gene expression (predominantly genes involved in metabolism) in liver tissues (Bannon et al., 2009a); however, the relevance of this finding to the carcinogenicity of RDX is unclear.

Sweeney et al. (2012b) hypothesized a set of MOAs for the liver tumors:

- *Genotoxicity mediated by either: (1) RDX; (2) tissue-generated oxidative metabolites; or (3) N-nitroso metabolites generated anaerobically in the GI tract.* The key events in this hypothesized MOA are: production of DNA damage, gene mutation, formation of neoplastic lesions, and promotion/progression of tumors. The largely negative results for genotoxicity led Sweeney et al. (2012b) to conclude that this MOA is not plausible for RDX or its

oxidative metabolites. Although there are some positive results for the N-nitroso metabolites, the limited evidence to support systemic uptake and distribution of metabolites to the liver led Sweeney et al. (2012b) to conclude that this MOA is not sufficiently plausible.

- *Cell proliferation.* The key events in this hypothesized MOA are GI-tract generation of N-nitroso metabolites, absorption, distribution to the liver, cytotoxicity (optional), and enhanced cell proliferation, leading to preneoplastic foci that progress to hepatocellular adenomas and carcinomas. Sweeney et al. (2012b) cited evidence of increased liver weights in mice as consistent with cell proliferation, but noted that increased liver weights were also observed in rats without proceeding to liver tumors. They considered this MOA "plausible, but not particularly well supported."

In addition to the inconsistencies in the evidence identified by Sweeney et al. (2012b), EPA notes the following evidence (or lack of evidence) that fails to support this hypothesized MOA. (1) The absence of significant liver histopathology in mice after subchronic or chronic exposure to RDX at doses that induced liver tumors (Lish et al., 1984; Cholakis et al., 1980) suggests that cellular toxicity is not a precursor to these tumors. (2) As discussed in Section 1.2.4, changes in liver weight showed no consistent pattern across studies or sexes, and did not correlate with tumor response. (3) No studies were available that directly measured RDX-induced cell proliferation rates. (4) No information was available to rule out non-precancerous causes of liver weight increase.

In summary, the available evidence indicates that RDX is likely not mutagenic (see Appendix C, Section C.3.2), although anaerobically-derived N-nitroso metabolites have demonstrated some genotoxic potential. While these metabolites have been measured in the mouse (Pan et al., 2007b) and identified in minipigs (Musick et al., 2010; Major et al., 2007), they have not been identified in humans, and may not be the predominant metabolites of RDX. A MOA involving a proliferative response generated by tissue-derived oxidative metabolites of RDX has been proposed, but is not supported by the available data. In light of limited information on precursor events leading to the observed liver and lung tumor response in RDX-exposed rodents and lack of toxicokinetic information on RDX metabolites, neither a cell proliferative MOA nor a mutagenic N-nitroso metabolite MOA is supported. Thus, the MOA leading to the increased incidence of liver and lungs tumors is not known.

1.3. INTEGRATION AND EVALUATION

1.3.1. Effects Other Than Cancer

The majority of evidence for the health effects of RDX comes from oral toxicity studies in animals. The three epidemiology studies that document possible inhalation exposure are limited by various study design deficiencies, including inability to distinguish exposure to TNT (associated with liver and hematological system toxicity), inability to adequately characterize exposure levels, small sample sizes, and inadequate reporting. The single animal inhalation study identified in the

literature search had deficiencies (e.g., lack of a control and incomplete exposure information) that precluded its inclusion in this assessment (see literature search section).

The strongest evidence for a human health hazard following exposure to RDX is for nervous system effects. Toxicity studies in multiple animal species involving chronic, subchronic, and gestational exposures provide consistent evidence of nervous system effects following oral exposure. Effects included dose-related increases in seizures and convulsions, as well as observations of tremors, hyperirritability, hyper-reactivity, and other behavioral changes (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a, b; Cholakis et al., 1980; von Oettingen et al., 1949).

Human studies provide supporting evidence for RDX as a neurotoxicant and provide support for the assumption that the nervous system effects observed in experimental animals are relevant to humans. In particular, several case reports provide evidence of associations between exposure to RDX (via ingestion, inhalation, and possibly dermal exposure) and seizures and convulsions (Kasuske et al., 2009; Küçükardali et al., 2003; Testud et al., 1996a; Testud et al., 1996b; Woody et al., 1986 and others, see Appendix C.2). Other nervous system effects identified in human case reports include dizziness, headache, confusion, and hyperirritability. A cross-sectional study described memory impairment and visual-spatial decrements in RDX-exposed workers (Ma and Li, 1993), although confidence in these findings is relatively low because of issues with design and reporting.

Additional support for an association between RDX exposure and nervous system effects comes from consistent evidence of neurotoxicity across taxa, including humans, laboratory animal species, birds, lizards, fathead minnows, and earthworms (Quinn et al., 2013; Garcia-Reyero et al., 2011; Mcfarland et al., 2009; Gogal et al., 2003). Studies in rats demonstrate a correlation between blood and brain concentrations of RDX and the time of seizure onset (Williams et al., 2011; Bannon et al., 2009a). Additionally, the affinity of RDX for the picrotoxin convulsant site of the $GABA_A$ channel suggests that the resulting disinhibition could lead to the onset of seizures (Williams et al., 2011).

Induction of convulsions and seizures appears to be more strongly correlated with dose than with duration of exposure. However, there is mechanistic information that suggests repeated binding to the receptor convulsant site of $GABA_A$ may promote a state of increased neuronal activity that increases the likelihood of subsequent neurological effects (Gerkin et al., 2010). As a result, some uncertainty remains as to whether the available mechanistic information adequately addresses potential neurotoxicity after longer-duration exposure to RDX. It is unclear if nervous system effects progressed in severity (e.g., from subtle behavioral changes or nonconvulsive seizures to tonic-clonic seizures) with increasing dose, as many of the studies that reported more subtle neurobehavioral changes did not provide detailed dose-response information, and the majority of studies were not designed to capture this information.

The nervous system effects following oral exposure to RDX were observed in humans acutely exposed to RDX and in multiple experimental animal studies in rats, mice, monkeys, and dogs following exposures ranging from 10 days to 2 years in duration. Notably, despite the potential for effects on the developing nervous system based on the presumed MOA for RDX neurotoxicity (discussed in Section 1.2.3), no studies included a thorough evaluation of potential developmental neurotoxicity. Across the database, behavioral manifestations of seizure activity were the most consistently observed nervous system effect associated with RDX exposure. This most commonly included evidence of increased convulsions, as well as other related effects such as tremors, shaking, hyperactivity, or nervousness, which were generally observed at doses that were the same as or higher than doses that induced convulsions. Nervous system effects are a human hazard of RDX exposure and are carried forward for consideration for dose-response analysis. Convulsions, considered a severe adverse effect, were selected as a consistent and sensitive endpoint representative of nervous system effects.

Evidence for urinary system toxicity is more limited than evidence for neurotoxicity. In humans, kidney effects (including decreased urine output, blood in urine, and proteinuria) were observed only in individuals with acute accidental exposure (ingestion and inhalation) to unknown amounts of RDX. In experimental animal studies, histopathological changes in the kidney and urinary bladder (medullary papillary necrosis, suppurative pyelitis, and uremic mineralization of the kidney; luminal distention and cystitis of the urinary bladder) were reported in male rats exposed to RDX in the diet following exposure durations of 1 year or longer (Levine et al., 1983), but not in similarly exposed female rats. Evidence for milder renal effects reported in subchronic studies of RDX in mice, rats, and monkeys was limited and inconsistent. Mice appeared to be less sensitive than rats. Other measures of kidney effects (kidney weights and serum chemistry parameters) did not provide consistent evidence of dose-related changes associated with RDX exposure. In light of the dose-related increase in histopathological changes in the kidney and urinary bladder in male rats in the Levine et al. (1983) study, and in particular the robust response in the high-dose animals, urinary system effects are a potential human hazard of RDX exposure.

Medullary papillary necrosis was selected as an endpoint representative of kidney effects. This histopathologic lesion was observed at higher incidence than other kidney histopathologic lesions, was present at both the 12-month interim and 2-year final sacrifices (Table 1-6), and represents a severe measure of toxicity. Renal toxicity was, in fact, considered the principal cause of RDX-related mortality and morbidity in male rats in the Levine et al. (1983) 2-year bioassay. Hemorrhagic/suppurative cystitis was selected as an endpoint representative of urinary bladder effects. Like medullary papillary necrosis of the kidney, urinary bladder cystitis is a clearly adverse effect and was observed at both the 12-month interim and 2-year final sacrifices (Table 1-7). A dose-related increased incidence of luminal distention was also observed in male rats, but was not selected as representative of urinary bladder toxicity because it is a less specific diagnosis than cystitis and can be caused by various factors, including partial obstruction of the bladder.

Evidence for prostate toxicity is also more limited than evidence for neurotoxicity. A dose-related, increase in the incidence of suppurative prostatitis was observed in male rats exposed to RDX in the diet for 2 years (Levine et al., 1983). There was also a concomitant shift from chronic inflammation to suppurative inflammation with increasing dose of RDX starting at 1.5 mg/kg-day. Similar types and patterns of inflammation were not observed in mice, and no other rat studies of equivalent duration that examined the prostate were available. RDX and its interaction with $GABA_A$ receptors, which have also been identified on the prostate (Napoleone et al., 1990), increases biological plausibility by providing a potential mechanism by which RDX could have effects directly on the prostate. In their evaluation of the external review draft assessment, the SAB determined the weight-of-evidence to be sufficient for identifying prostate effects as a hazard of RDX exposure (SAB, 2017). Consistent with this determination, the incidence of suppurative prostatitis was selected as the endpoint most representative of prostate effects.

Evidence for developmental toxicity and liver toxicity was more limited than that for the endpoints discussed above. In animal studies, developmental effects, including offspring survival, growth, and morphological development, were observed only at doses associated with maternal mortality (Angerhofer et al., 1986; Cholakis et al., 1980). Evidence for potential hepatic effects comes from observations of increases (generally dose-related) in liver weight in some subchronic oral animal studies (Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a, b; Cholakis et al., 1980; Hart, 1976). However, these elevations in liver weight were not consistently observed across studies nor were they accompanied by RDX-related histopathological changes in the liver or increases in serum liver enzymes. In addition, the interpretation of liver weight changes in the mouse bioassay by Lish et al. (1984) is complicated by the relatively high incidence of liver tumors in this study. At this time, the available data do not support liver and developmental toxicity as human hazards of RDX exposure; these effects were not considered further for dose-response analysis and derivation of reference values.

Evidence that RDX may induce effects in other organs, including the eyes, and the cardiovascular, musculoskeletal, immune, GI, hematological, and reproductive systems, is generally limited to human case reports or to findings in experimental animals that were inconsistent across studies or lacked dose-related patterns of response. Therefore, information to assess toxicity in these organs following exposure to RDX was insufficient. Treatment-related changes in body weight or body weight gain were generally observed at high doses in association with elevated mortality or with severe kidney and urinary bladder toxicity, and thus appeared to be secondary to effects on other primary targets of RDX toxicity. Effects on body weight and on these other organs were not considered further for dose-response analysis and derivation of reference values.

In a number of the animal studies reporting nervous system effects, unscheduled deaths occurred at RDX doses as low as those that induced nervous system effects (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a; Cholakis et al., 1980; von Oettingen et al., 1949). In a 90-day study that recorded nervous system effects and survival more thoroughly

than earlier studies, Crouse et al. (2006) reported that nearly all pre-term deaths were preceded by neurotoxic signs such as tremors and convulsions. Convulsions did not, however, necessarily lead to early mortality; of the animals observed to have convulsed in the Crouse et al. (2006) study, approximately 75% survived to the end of the 90-day study. Most of the earlier studies provide a limited understanding of the association between mortality and nervous system effects because the frequency of clinical observations was likely insufficient to observe convulsions prior to death. In humans, mortality has not been reported in case reports involving workers with symptoms of neurotoxicity exposed to RDX during manufacture or in individuals exposed acutely as a result of accidental or intentional ingestion. Survival has not been specifically evaluated in studies of worker populations exposed chronically to RDX. Ultimately, the convulsion findings, without consideration of mortality, are sufficient to identify neurotoxic effects associated with RDX exposure as severe and adverse.

Regarding mortality, the preference is not to use a frank health effect as severe as mortality as the basis for a reference value. As noted in U.S. EPA (2002), a chemical may cause a variety of effects ranging from severe—such as death—to more subtle biochemical, physiological, or pathological changes; primary attention in assessing health risk should be given to those effects in the lower exposure range and/or the effects most biologically appropriate for a human health risk assessment. Where mortality occurs as a consequence of a chemical's effects on a specific organ/system (e.g., in the case of RDX, evidence suggests some relationship between mortality and effects on the nervous system and kidney), the preference would be to develop a quantitative assessment based on the initial hazard and not on death. Because unscheduled deaths were observed with some consistency across studies and, in some studies, at doses as low as those associated with convulsions, two additional analyses of mortality data are presented in Chapter 2. In the first analysis, BMDs derived using mortality data sets are compared to the BMD used to derive the RfC (Section 2.1.6). As discussed in Section 1.2.1, the relationship between convulsions and mortality is not clear and raises concerns for the potential underreporting of convulsions. An analysis, described in Section 2.1.7, addresses the possibility that the analyses of convulsions brought forward for dose-response analysis resulted in an underestimate of the toxicity for RDX.

1.3.2. Carcinogenicity

As presented in Section 1.2.7, dietary administration of RDX induced dose-related increases in the incidence of hepatocellular adenomas or carcinomas in male and female B6C3F$_1$ mice (Parker et al., 2006; Lish et al., 1984). In the same study, RDX also induced dose-related increases in the incidence of alveolar/bronchiolar adenomas or carcinomas in both sexes. Some of these trends in liver and lung were statistically significant. In Fischer 344 rats, dietary administration of RDX

yielded a statistically significant trend in the incidence of hepatocellular carcinomas[24] in males, but not in females (Levine et al., 1983). A 2-year dietary study in Sprague-Dawley rats was negative in both sexes Hart (1976), although the highest dose in this study, and the only dosed group for which pathology was examined, was somewhat lower (no increase in carcinomas at doses up to 10 mg/kg-day in Hart (1976), versus hepatocellular carcinomas in male rats at 8 and 40 mg/kg-day in the Levine et al. (1983) study). The human studies are not informative.

This evidence leads to consideration of two hazard descriptors under the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). The descriptor *likely to be carcinogenic to humans* is appropriate when the evidence is "adequate to demonstrate carcinogenic potential to humans" but does not support the descriptor *carcinogenic to humans*. One example from the cancer guidelines is "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." RDX matches the conditions of this example, having induced dose-related increases in tumors in two species (mouse and rat), in both sexes, and at two sites (liver and lung). Liver carcinomas, increased in male F344 rats in the Levine et al. (1983) study, are considered rare in that species.

Alternatively, the descriptor *suggestive evidence of carcinogenic potential* is appropriate when the evidence raises "a concern for potential carcinogenic effects in humans" but is not sufficient for a stronger conclusion. The incidences of alveolar/bronchiolar tumors showed a positive trend in male and female $B6C3F_1$ mice. Evidence of carcinogenicity in the liver from rodent bioassays is less clear. The hepatocellular carcinoma result in male F344 rats is based on a small number of tumors (1/55, 0/55, 0/52, 2/55, and 2/31, respectively, at 0, 0.3, 1.5, 8.0, and 40 mg/kg-day) that is not matched by an increase in hepatocellular neoplasms overall (5/55, 3/55, 0/52, 4/55, and 3/31, respectively), and RDX did not increase the incidence of carcinomas at any other site in F344 or Sprague-Dawley rats of either sex. The incidence of liver tumors in female $B6C3F_1$ mice showed a statistically significant positive trend (Lish et al., 1984), although the authors noted the relatively low tumor incidence in concurrent female control mice (1/65). The PWG that reviewed the slides from this study (Parker et al., 2006; Parker, 2001) confirmed the positive trend in female mouse liver tumors, but also raised some concerns related to histopathological methods and the absence of necropsy and histopathology processing records that limited their evaluation. In male mice from this study (Lish et al., 1984), the incidences of liver tumors in some treated groups were higher than in the control, but trend tests were not statistically significant. Interpretation of male mouse liver tumor incidence is complicated by the high and variable background incidence of this tumor in the male mouse.

[24]Hepatocellular carcinoma may be regarded as a rare tumor in male F344 rats. Although there is no compilation of historical control data for the Levine laboratory, Haseman et al. (1984) reported that in NTP studies during 1980–1983, 18/2306 (0.8%) of male F344 rats developed hepatocellular carcinomas and 78/2306 (3.4%) developed neoplastic nodules.

As discussed in Section 1.2.7, few mechanistic studies are available to inform the mode of action by which RDX induces liver and lung tumors in rodents. The available evidence indicates that RDX is likely not mutagenic. Anaerobically-derived N-nitroso metabolites have demonstrated some genotoxic potential. These metabolites have not been identified in humans, and their contribution to any genotoxic potential of RDX is unknown. Precursor events leading to the observed liver and lung tumor response in RDX-exposed rodents have not been identified. Although characterization of the cancer MOA is not needed to determine a chemical's cancer hazard, understanding the MOA can contribute to a cancer hazard determination. In the case of RDX, mechanistic information is not helpful in guiding selection of a cancer descriptor.

As noted in the EPA's cancer guidelines (U.S. EPA, 2005a), choosing a hazard descriptor cannot be reduced to a formula, as descriptors may be applicable to a variety of potential data sets and represent points along a continuum of evidence. In the case of RDX, there are plausible scientific arguments for more than one hazard descriptor. Overall, the considerations discussed above, interpreted in light of the cancer guidelines, lead to the conclusion that there is *suggestive evidence of carcinogenic potential* for RDX. Although the evidence includes dose-related tumor increases in two species, two sexes, and two sites, the evidence of carcinogenicity outside the $B6C3F_1$ mouse is not robust, and this factor was decisive in choosing a hazard descriptor. Within the spectrum of results covered by the descriptor *suggestive evidence*, the evidence for RDX is strong. There are well-conducted studies that tested large numbers of animals at multiple dose levels, making the cancer response suitable for dose-response analysis (Section 2).

The descriptor *suggestive evidence of carcinogenic potential* applies to all routes of human exposure. Dietary administration of RDX to mice and rats induced tumors of the liver or lung, sites beyond the point of initial contact, and human case reports have demonstrated absorption and distribution of inhaled RDX into systemic circulation. Under the cancer guidelines, this information provides sufficient basis to apply the cancer descriptor developed from oral studies to other exposure routes.

1.3.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

Susceptibility refers to factors such as lifestage, genetics, sex, and health status that may predispose a group of individuals to greater response to an exposure. This greater response could be achieved either through differences in exposure to the chemical underlying RDX toxicokinetics or differences in RDX toxicodynamics between susceptible and other populations. Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Reproductive and developmental toxicity studies generally did not identify effects in offspring at doses below those that also caused severe maternal toxicity (Angerhofer et al., 1986; Cholakis et al., 1980). However, the developmental importance of GABAergic systems (Kirmse et al., 2018; Ben-Ari, 2014; Williams et al., 2011; Williams and Bannon, 2009; Galanopoulou, 2008) and developmental neurotoxicity of chemicals with similar modes of action (Salari and Amani, 2017; Marty et al., 2000) suggest RDX may be harmful during the period of brain development. Further

raising cause for concern, seizures and seizure disorders such as epilepsy occur more frequently in infants and children than in any other age group (many are caused by early-life insults such as fever or trauma), and research suggests that early life seizures (i.e., before the brain has fully matured) can lead to long-lasting neurological consequences (Ronnie, 2003; Volpe, 2001; Jensen and Baram, 2000; Moshé, 2000, 1987). A pilot study in rats demonstrated transfer of RDX from dam to fetus during gestation, found RDX in milk from treated dams, and recommended further study (Hess-Ruth et al., 2007). Given the understanding of RDX toxicokinetics (see Section 1.1.2), it is expected that RDX reaching the fetus or infant through either the blood or ingested milk would be readily distributed to the brain, although specific studies have not been conducted. For these reasons, and as noted in Section 1.2.1, the lack of developmental neurotoxicity studies was identified as a significant data gap in understanding the nervous system effects of RDX exposure.

The primary MOA for the neurotoxic effects of RDX exposure involves RDX binding to $GABA_A$ receptors, specifically the picrotoxin convulsant site of the GABA channel, and blocking inhibitory GABAergic transmission, that eventually leads to the development of seizures and related behavioral changes (see Section 1.2.1, Mechanistic Evidence). In addition to its role as the major inhibitory neurotransmitter system in many regions of the adult brain, GABAergic signaling plays a key role in brain development, where it contributes to a delicate equilibrium with other signaling processes (e.g., glutamatergic) to help establish the appropriate functional connectivity of the mature brain (Kirmse et al., 2018; Ben-Ari, 2014). While GABAergic signaling and the overall balance between excitation and inhibition is essential throughout brain development, which continues through sexual maturation, a number of critical developmental processes occur simultaneously during the perinatal period, and these coincide with prominent shifts in GABAergic function. As a result, the perinatal period may represent a vulnerable lifestage for the neurotoxic effects of RDX exposure through GABAergic inhibition.

In the perinatal mammalian brain, GABA activity is primarily depolarizing and excitatory (as compared to hyperpolarizing and inhibitory in the adult brain), which is presumably necessary for its specific functions at this stage of brain development. In animals, expression of chloride co-transporters NKCC1 and KCC2 around or shortly after birth reduces intracellular Cl^- and mediates a switch in GABA activity to primarily hyperpolarizing and inhibitory (Ben-Ari, 2014; Rivera et al., 1999). For $GABA_A$ergic signaling, the switch from depolarizing to the hyperpolarizing phenotype in adults occurs by the end of the first postnatal month in rats, although this differs by brain region and sex (Galanopoulou, 2008). In addition, the composition of $GABA_A$ receptors is also subject to developmental regulation, with some subunits varying in their pattern of expression during development as compared to adulthood (Luján et al., 2005; Fritschy et al., 1994; Laurie et al., 1992). Thus, RDX exposure during the perinatal period in humans could be impactful.

During this potentially sensitive period, excitatory $GABA_A$ergic signaling helps to regulate the proliferation, migration, survival, and differentiation of new neurons, as well as synaptogenesis and the development of mature neural networks (Deidda et al., 2014; Galanopoulou, 2008).

Modulation of $GABA_A$ergic signaling at this lifestage is presumably tightly controlled, as it serves to orchestrate these processes in a region-specific manner for specific glial and neuronal subsets, often stimulating these processes (e.g., increasing neuronal migration or survival) in some regions while simultaneously inhibiting the same processes (e.g., decreasing neuronal migration or survival) in other regions (Creeley, 2016; Deidda et al., 2014; Galanopoulou, 2008; Ikonomidou et al., 2000). Additional concern for susceptibility during this lifestage may be raised due to the prominent role for BDNF during this time, with high expression during the first two postnatal weeks (in rodents) before declining to adult levels, and whose role as a neurotrophic factor includes the regulation of neuronal excitation and its sequelae (Aguado et al., 2003). As discussed in Section 1.2.1, molecular evidence suggests that RDX exposure in adults may impact the expression or function of BDNF and related factors in the brain (Zhang and Pan, 2009b); the lack of data on brain BDNF after developmental RDX exposure remains a data gap.

Alterations of GABA activity have been linked to developmental brain disorders (Kirmse et al., 2018) and genetic mutations causing aberrant GABAergic signaling lead to a number of seizure disorders in infants and children (Galanopoulou, 2008), although GABAergic signaling in the immature brain may be required for epileptogenesis (Khalilov et al., 2003). Exposure of early postnatal rodent hippocampus to the $GABA_A$ receptor antagonist, bicuculline, which has a similar mode of action to RDX, increased the density of inhibitory but not excitatory synapses (Marty et al., 2000). White et al. (2008) reported that a 2.7 mg/kg subcutaneous dose of bicuculline provoked seizures in 97% adult mice, but Salari and Amani (2017) found developmental and behavioral impairment after a 0.3 mg/kg subcutaneous dose to neonatal mice, suggesting developmental neurotoxicity from bicuculline is evident as low as 1/10 the convulsive dose. Findings from studies of bicuculline provide suggestive evidence of perinatal susceptibility to the neurotoxicity elicited by compounds that alter GABAergic signaling. The lack of data on how RDX exposure might impact the critical role of GABAergic signaling during the perinatal period (and at later stages of brain development and maturation) represents an important uncertainty.

Limited data suggest that male laboratory animals may be more susceptible to noncancer toxicity associated with RDX exposure. In general, male animals were more sensitive to RDX neurotoxicity than females (i.e., more convulsions; more hyperactive; greater brain weight changes). In the two-year study in F344 rats (Levine et al., 1983), RDX exposure induced severe toxicity of the kidney and urinary bladder in males, but no similar effects in females, suggesting a sex-based difference in susceptibility to RDX urinary system toxicity.

Data on the incidence of convulsions and mortality from gavage studies of RDX in the rat provide some indication that pregnant animals may be a susceptible population. In the developmental toxicity study by Cholakis et al. (1980), deaths were observed in pregnant F344 rats only at a dose of 20 mg/kg-day, but convulsions were reported in a single rat at 2 mg/kg-day. In a range-finding developmental toxicity study (Angerhofer et al., 1986), mortality and convulsions were reported in pregnant Sprague-Dawley rats at a dose of ≥40 mg/kg-day, but not at ≤20 mg/kg-

day, although the relatively small group sizes in this study should be noted. In the main study by these investigators, convulsions were reported in pregnant rats only at 20 mg/kg-day, but one death (in dose groups of 40 rats) was reported at both 2 and 6 mg/kg-day (Angerhofer et al., 1986). In comparison, increased mortality and convulsions were reported at ≥8 mg/kg-day in a 90-day gavage study in F344 rats (Crouse et al., 2006). The instances of one convulsion and two deaths in pregnant rats in the Cholakis et al. (1980) and Angerhofer et al. (1986) studies at doses of 2 or 6 mg/kg-day raise the possibility that pregnant animals may be more susceptible to the effects of RDX; however, direct comparison between the available gavage studies in pregnant and nonpregnant rats is uncertain because of differences in study design, including numbers of animals tested per group, test material characteristics, and rat strain. Overall, the available information is not considered sufficient to conclude that pregnant animals are a susceptible population.

There is limited evidence that CYP450 or similar enzymes are involved in the metabolism of RDX (Bhushan et al., 2003), indicating a potential for genetic polymorphisms in these metabolic enzymes to affect susceptibility to RDX. This susceptibility may also be influenced by differential expression of these enzymes during development. Individuals with epilepsy or other seizure syndromes, and in particular those that have their basis in genetic mutation to $GABA_A$ receptors, may represent another group that may be susceptible to RDX exposure. However, there is currently no information to support predictions of how genetic polymorphisms or the presence of seizure syndromes may affect susceptibility to RDX exposure.

2. DOSE-RESPONSE ANALYSIS

2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The oral reference dose (RfD, expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

2.1.1. Identification of Studies for Dose-Response Analysis of Selected Effects

As discussed in Section 1.3.1, based on findings from oral studies in experimental animals, nervous system effects are a human hazard of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure, and urinary system (kidney and bladder) effects are a potential human hazard of RDX exposure. There is suggestive evidence of prostate effects associated with RDX exposure. Although animal mortality has been reported in a number of the toxicology studies conducted for RDX, it was not considered a hazard by itself or as the basis for the derivation of a reference value (see Sections 2.1.6 and 2.1.7 for further discussion).

The effects selected to best represent each of the hazards, identified in Section 1.3.1, are carried forward in the sections below. In order to identify the stronger studies for dose-response analysis, several attributes of the studies reporting the endpoints selected for each hazard were reviewed (i.e., study size and design, relevance of the exposure paradigm, and measurement of the endpoints of interest). In considering the study size and design, preference was given to studies using designs reasonably expected to have power to detect responses of suitable magnitude. Exposure paradigms including a route of human environmental exposure (i.e., oral and inhalation) are preferred. When developing a chronic reference value, chronic or subchronic studies are preferred over studies of acute exposure durations. Studies with a broad exposure range and multiple exposure levels are preferred to the extent that they can provide information about the shape of the exposure-response relationship. Additionally, with respect to measurement of the endpoint, studies that can reliably distinguish the presence or absence (or degree of severity) of the effect are preferred.

Human studies are generally preferred over animal studies as the basis for a reference value when quantitative measures of exposure are reported, and the reported effects are determined to be associated with exposure. The available epidemiological studies of worker populations exposed

to RDX examined the relationship between certain health endpoints and inhalation exposure; however, no epidemiological studies of ingested RDX are available. Multiple case reports support the identification of hazards associated with RDX exposure but are inadequate for dose-response analysis because they do not yield incidence estimates, exposure durations are short, and quantitative exposure information is lacking. Therefore, human studies could not be used for oral dose-response analysis or to serve as the basis for the RfD. In the absence of human data, the animal studies were considered for dose-response analysis.

Experimental animal studies considered for each health effect were evaluated using general study quality considerations discussed in Section 4 of the Preamble and in the literature search section, and the attributes described above. The rationales for selecting the strongest studies that reported effects on the nervous system, urinary system, and prostate are summarized below.

Nervous System Effects

Convulsions, a severe adverse effect, were selected for dose-response analysis as a consistent endpoint of nervous system effects (see Section 1.3.1 for discussion). This endpoint was reported in seven studies (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Cholakis et al., 1980; Martin and Hart, 1974; von Oettingen et al., 1949). Table 2-1 provides an overview of the information considered in the studies reporting nervous system effects (i.e., convulsions) evaluated for dose-response analysis.

Table 2-1. Information considered for evaluation of studies that examined convulsions

Study reference	Study design and size		Exposure paradigm						Measurement of endpoint
	Design	# of animals	Route	Duration	# of dose groups[a]	Levels (mg/kg-d)	Purity (%)	Analytical conc?[b]	Incidence data reported
Crouse et al. (2006)	Toxicity study	10 rats/ sex/ group	Gavage	13-wk	5	4–15	99.99	Yes	Yes
Cholakis et al. (1980)	Developmental study	24–25 female rats/group	Gavage	14-d	3	0.2–20	89	Yes	Yes
Martin and Hart (1974)	Toxicity study	3 monkeys/ sex/group	Gavage	13-wk	3	0.1–10	Not specified	Not specified	Yes
Levine et al. (1983)	Toxicity and carcinogenicity bioassay	75 rats/ sex/group	Diet	2-yr	4	0.3–40	89–99	Yes	No
Lish et al. (1984)	Toxicity and carcinogenicity bioassay	85 mice/ sex/group	Diet	2-yr	4	1.5–175	89–99	Yes	No
Levine et al. (1981a)	Toxicity study	10 rats/ sex/group	Diet	13-wk	5	10–600	85	Yes	No
von Oettingen et al. (1949)	Toxicity study	20 rats/ group	Diet	13-wk	3	15–50	90–97	Not specified	No

[a]Excluding the control group.
[b]Indicates whether authors performed analysis to confirm the concentration of RDX in the suspension or diet administered to the animals (e.g., to determine percentage of target concentration).

Incidence of convulsions was reported in three studies of RDX—all involving gavage administration: Crouse et al. (2006), Cholakis et al. (1980) (developmental toxicity study), and Martin and Hart (1974). Qualitative findings of nervous system effects were reported in other chronic and subchronic studies—all involving dietary administration: Lish et al. (1984), Levine et al. (1983), Levine et al. (1981a), and von Oettingen et al. (1949). Incidence data on neurotoxic effects of RDX were not collected in any of the dietary studies. For example, Levine et al. (1983) reported only that convulsions and other nervous system effects were noted in rats exposed to RDX for 2 years at the highest dose (40 mg/kg-day) tested. The studies that included incidence data (i.e., the gavage studies) were preferred over those studies only reporting qualitative results (i.e., the dietary studies).

The three gavage studies reporting incidence data were further considered. Crouse et al. (2006) reported a dose-related increase in convulsions and tremors in both male and female F344

rats following a 90-day oral (gavage) exposure to RDX. This study used a test material of high purity and six dose groups (including the control) that provided good resolution of the dose-response curve. Cholakis et al. (1980) reported a dose-related increase in convulsions in a developmental toxicity study in F344 rats, following a 14-day exposure to RDX on gestational days (GDs) 6–19. Although this study was designed as a standard developmental toxicity study (i.e., not specifically to examine nervous system effects), it reported information on the identity of the test material and used three dose groups that adequately characterized the dose-response curve. Further, this study provided evidence of nervous system effects at a relatively low dose. The study in monkeys by Martin and Hart (1974) provides supporting evidence of nervous system effects (trembling, shaking, ataxia, hyperactive reflexes, and convulsions); however, this study was not selected for dose-response analysis because of small group sizes (n = 3/sex) and uncertainty in measures of exposures (e.g., purity of the test material was not specified, and reported emesis in some animals likely influenced the delivered dose).

Although the gavage studies reporting incidence data were preferred over four dietary studies (Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; von Oettingen et al., 1949) that did not provide incidence data, it is important to note that the reported neurotoxic effects in the dietary studies were observed at dose levels higher than the doses at which effects were observed in the gavage studies (Crouse et al., 2006; Cholakis et al., 1980; Martin and Hart, 1974). Given this potential difference based on dosing method, the dietary studies were also considered for quantitative analysis, despite the lack of incidence data, to evaluate the influence of oral dosing method on candidate values. In the 2-year study by Levine et al. (1983), a LOAEL for nervous system effects (convulsions, tremors, and hyper-irritability) of 40 mg/kg-day and a NOAEL of 8 mg/kg-day were identified. Other studies identified higher effect levels (i.e., 100 mg/kg-day in the 2-year mouse study by Lish et al. (1984) and 50 mg/kg-day in the 3-month rat study by von Oettingen et al. (1949)), and, with the exception of Lish et al. (1984), used shorter exposure durations. The unusual dosing regimen in the Cholakis et al. (1980) 13-week mouse study precluded identification of a NOAEL and LOAEL, and the single-dose design of the 6-week dog study by von Oettingen et al. (1949) did not allow identification of a NOAEL. As discussed in Section 1.2.1 and Table 1-3, the technical report of the 13-week study by Levine et al. (1981a) inconsistently identified the dose level at which convulsions occurred; therefore, a reliable NOAEL and LOAEL from this study could not be identified.

Therefore, two gavage studies, Crouse et al. (2006) and Cholakis et al. (1980), and one dietary study, Levine et al. (1983), were selected for dose-response analysis.

Urinary System (Kidney and Bladder) Effects

Medullary papillary necrosis and hemorrhagic/suppurative cystitis were selected for dose-response analysis as biologically significant measures of kidney and urinary bladder effects, respectively (see Section 1.3.1 for discussion). These histopathologic lesions of the urinary system were primarily observed in the 2-year study by Levine et al. (1983). Levine et al. (1983) included

histopathologic examination of kidney and bladder tissues at 6-, 12-, and 24-month time points; included four dose groups and a control group, and adequate numbers of animals per dose group (75/sex/group, with interim sacrifice groups of 10/sex/group at 6 and 12 months); and reported individual animal data. Other studies in rats using subchronic exposure durations or lower dose levels did not observe similar effects on the urinary system as did Levine et al. (1983), and studies in mice suggest that this species is less sensitive to RDX toxicity on the urinary system. Therefore, incidence data from the 2-year dietary study by Levine et al. (1983) were selected for dose-response analysis.

Prostate Effects

Suppurative prostatitis as reported in male rats in the Levine et al. (1983) study was selected for dose-response analysis as a biologically significant measure of prostate effects (see Section 1.3.1 for discussion). This study included histopathologic examination of the prostate at 6-, 12-, and 24-month time points; included four dose groups and a control group, and adequate numbers of animals per dose group (75/sex/group, with interim sacrifice groups of 10/sex/group at 6 and 12 months); and reported individual animal data. Levine et al. (1983), the only study to identify an increased incidence of suppurative prostatitis associated with RDX exposure, was selected for dose-response analysis.

2.1.2. Methods of Analysis

No biologically based dose-response models are available for RDX. In this situation, the U.S. Environmental Protection Agency (EPA) evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, EPA evaluated dose-response information with the models available in EPA's Benchmark Dose Software (BMDS, versions 2.4 and 2.5). EPA estimated the benchmark dose (BMD) and BMDL using a benchmark response (BMR) selected for each effect. A conceptual model of the analysis approach used for RDX is provided in Figure 2-1. In this assessment, points of departure (PODs) are identified through BMD modeling (preferred) or identification of a NOAEL, and followed by animal-to-human extrapolation using physiologically based pharmacokinetic (PBPK) models or the application of a dosimetric adjustment factor, depending on the data available.

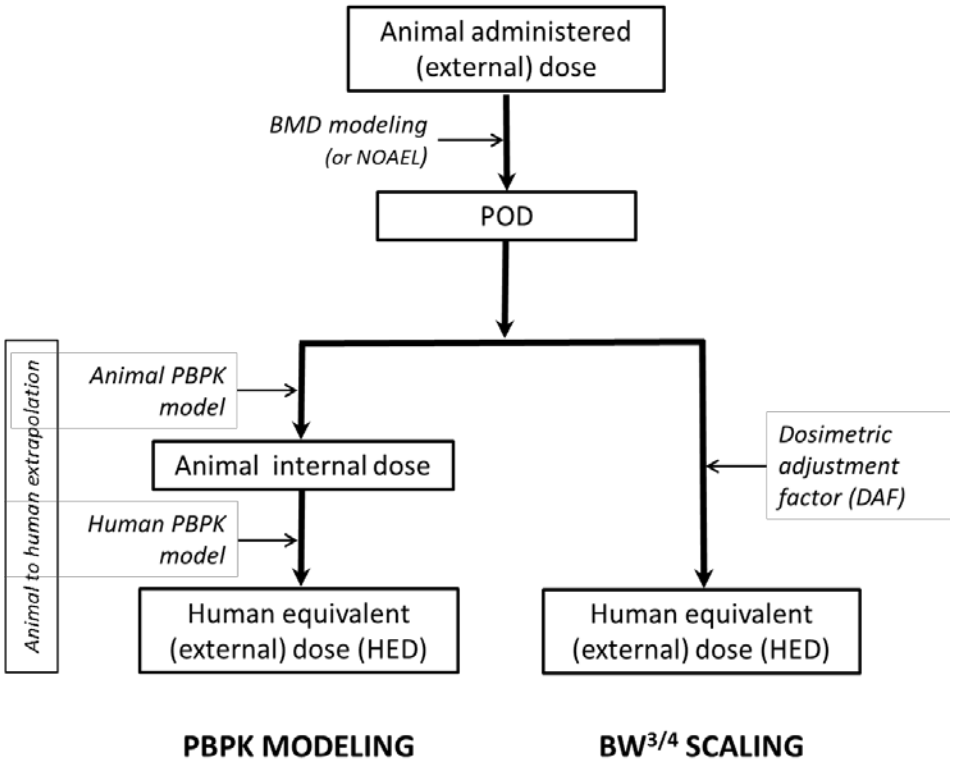


Figure 2-1. Conceptual approach to dose-response modeling for oral exposure.

Nervous System Effects

Incidence data for convulsions from Crouse et al. (2006) and Cholakis et al. (1980) were amenable to BMD modeling. For Crouse et al. (2006), statistical analysis conducted by EPA indicated no significant difference in convulsion rates of male and female rats (exact Wald-type chi-square test, accounting for dose; see Table 2-2); thus, combined incidence data from male and female rats were used for modeling convulsion data from this study.

In general, there is a strong preference to use a less severe endpoint as the basis for a noncancer toxicity value. As discussed in the evaluation of nervous system effects (Section 1.2.1), evidence from other seizurogenic compounds with modes of action similar to RDX suggests that other generally subclinical cognitive and behavioral neurological effects are likely to occur at lower RDX doses, although limited investigation has been conducted to establish such subclinical effects.

EPA guidelines indicate that a BMR of 5% or lower may be warranted for frank effects (such as convulsions) (U.S. EPA, 2012a). EPA considered BMRs of 1 and 5% extra risk (ER) for convulsions. A BMR of 1% ER was considered appropriate to address the severity of convulsions, a frank effect. The use of a 1% ER BMR for convulsions in Crouse et al. (2006) resulted in extrapolation below the range of the experimental doses. More specifically, the BMD of 3.02

mg/kg-day with a 1% BMR was below the LOAEL of 8 mg/kg-day (with a 15% response rate for convulsions), although the BMD was not far below the dose range of 4–15 mg/kg-day used in the study.

EPA considered the trade-off between (1) a BMR of 1% ER that addresses the severity of convulsions, but results in extrapolation outside the experimental range, and (2) a 5% ER BMR that may be inadequate for addressing the severity of these specific outcomes, but is more consistent with the available data. EPA selected a BMR of 5% ER, addressing the lack of incidence data for less severe endpoints through application of the database uncertainty factor (i.e., reflecting insufficient investigation of less severe, subclinical, nervous system effects for RDX). See Section 2.1.3, Derivation of Candidate Values, for further discussion of the database uncertainty factor.

Because incidence data for convulsions were not provided by Levine et al. (1983), a NOAEL was used as the POD for this dataset rather than a BMDL.

Table 2-2 summarizes the PODs derived for each data set. More detailed BMD modeling information is available in Appendix D; BMD and BMDL estimates for 1 and 10% ER for the selected model (see Appendix D, Section D.1.2, Tables D-3 and D-4) are provided for comparative purposes.

Urinary System (Kidney and Bladder) Effects

Incidence data for medullary papillary necrosis in the kidney (as reported by Levine et al. (1983)) was considered unsuitable for modeling. Aside from the lowest positive dose (which had incidence of 1/55), only the highest dose group had a positive response (18/31 or 58%), which was higher than a level of change considered to be minimally biologically significant (e.g., 10% ER). In this case, because there is insufficient information to estimate the BMD (U.S. EPA, 2012a), these data were not modeled. In the absence of sufficient information to conduct BMD modeling, a NOAEL of 8 mg/kg-day was used as the POD for this dataset (see Table 2-2).

Incidence data for hemorrhagic/suppurative cystitis in the urinary bladder as reported by Levine et al. (1983) were amenable to BMD modeling. The BMDS models were fit to these data using a BMR of 10% ER, under the assumption that it represents a minimally biologically significant level of change. Table 2-2 summarizes the POD derived using data on the incidence of hemorrhagic/suppurative cystitis. More detailed BMD modeling information is available in Appendix D, Section D.1.2, Table D-6.

Prostate Effects

Incidence data on suppurative prostatitis as reported by Levine et al. (1983) were amenable to BMD modeling. A BMR of 10% ER was applied under the assumption that it represents a minimally biologically significant level of change. Table 2-2 summarizes the POD derived using data on the incidence of suppurative prostatitis. More detailed BMD modeling information is available in Appendix D, Section D.1.2, Table D-7.

Table 2-2. Summary of derivation of PODs following oral exposure to RDX

Endpoint and reference (exposure duration/route)	Species/sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{HED} (mg/kg-d)		
						Administered dose[b]	RDX AUC[c]	RDX C_{max}[d]
Nervous system								
Incidence of convulsions Crouse et al. (2006) (90-d/gavage)	Male and female F344 rat, combined[e]	Multistage 3°	5% ER	5.19	2.66	0.64	1.3	1.7
Incidence of convulsions Cholakis et al. (1980) (GDs 6–19/gavage)	Female F344 rat	Quantal-linear	5% ER	0.915	0.628	0.15	0.31	0.41
Incidence of convulsions Levine et al. (1983) (2-yr/diet)	Male and female F344 rat	LOAEL = 40 mg/kg-d; NOAEL = 8 mg/kg-d[f]				1.9	3.9	4.3
Urinary system (kidney and bladder)								
Kidney: medullary papillary necrosis Levine et al. (1983) (2-yr/diet)	Male F344 rat	LOAEL = 40 mg/kg-d; NOAEL = 8 mg/kg-d[g]				1.9	3.9	4.3
Urinary bladder: hemorrhagic/ suppurative cystitis Levine et al. (1983) (2-yr/diet)	Male F344 rat	Multistage 3°	10% ER	20.0	11.6	2.8	5.6	6.3

Endpoint and reference (exposure duration/route)	Species/sex	Model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	POD_{HED} (mg/kg-d)		
						Administered dose[b]	RDX AUC[c]	RDX C_{max}[d]
Prostate								
Incidence of suppurative prostatitis Levine et al. (1983) (2-yr/diet)	Male F344 rat	LogProbit	10% ER	1.67	0.47	0.11	0.23	0.25

[a]For modeling details, see Appendix D.
[b]POD was converted to an HED using a standard DAF based on $BW^{3/4}$. See Section 2.1.2, Methods of Analysis/ Extrapolation using $BW^{3/4}$ scaling for DAFs.
[c]POD was converted to an HED based on the equivalence of internal RDX dose (expressed as AUC for RDX concentration in arterial blood) derived using PBPK models.
[d]POD was converted to an HED based on the equivalence of internal RDX dose (expressed as peak RDX concentration in arterial blood, C_{max}) derived using PBPK models.
[e]Exact Wald-type chi-square exact test for differences in convulsion incidence across sexes yielded *p*-value >0.05.
[f]Nervous system effects for male and female rats reported qualitatively; incidence of convulsions and other nervous system effects was not reported. Therefore, available data do not support BMD modeling.
[g]BMD modeling was not supported for this data set; see discussion in text.

AUC = area under the curve; BW = body weight; DAF = dosimetric adjustment factor; ER = extra risk; HED = human equivalent dose

Human Extrapolation

EPA guidance (U.S. EPA, 2011) describes a hierarchy of approaches for deriving human equivalent doses (HEDs) from data in laboratory animals, with the preferred approach being PBPK modeling. Other approaches can include using chemical-specific information in the absence of a complete PBPK model. In lieu of either reliable, chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., $BW^{3/4}$) approach is generally applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans.

Candidate PODs for endpoints selected from rat and mouse bioassays were expressed as HEDs. HEDs were derived using both PBPK modeling (with alternative measures of internal dose), and a $BW^{3/4}$ scaling approach. These approaches are outlined in Figure 2-1, and the resulting POD_{HED} values are presented in Table 2-2.

Extrapolation Using PBPK Modeling.

PBPK models for RDX in rats, humans, and mice have been published (Sweeney et al., 2012a; Sweeney et al., 2012b; Krishnan et al., 2009) based on RDX-specific data. EPA evaluated and further developed these models for extrapolating doses from animals to humans (see Appendix C,

Section C.1.5). In general, appropriately chosen internal dose metrics are expected to correlate more closely with toxic responses than external doses for effects that are not occurring at the point of contact (McLanahan et al., 2012). Therefore, PBPK model-derived arterial blood concentration of RDX is considered a better dose-metric for extrapolation of health effects than administered dose when there is adequate confidence in the estimated value. The PBPK models for RDX were used to estimate two dose metrics: the area under the curve (AUC) and the peak concentration (C_{max}) for RDX concentration in arterial blood. The AUC represents the average blood RDX concentration for the exposure duration normalized to 24 hours and the C_{max} represents the maximum RDX concentration for the exposure duration.

Ideally, use of RDX concentrations in the brain would serve as the internal dose metric for analyzing convulsion data. However, the blood concentration of RDX was preferred as the dose metric due to greater confidence in modeling this variable. This is because of the substantially greater number of measurements of RDX blood levels used in calibrating model parameters. Additionally, predictions of RDX concentrations in the brain are highly correlated with predictions of RDX blood concentrations, since the model is flow-limited and no metabolism is assumed in that organ. Greater confidence was placed in model estimates of blood AUC than peak blood concentrations because, as discussed in Appendix C, Section C.1.5, the rate constant for oral absorption (KAS) is uncertain, and peak concentrations are more sensitive to variations in this parameter than average values. RDX-induction of convulsions and seizures appears to be more strongly correlated with dose than exposure duration, which might argue for use of peak blood concentration as an appropriate dose metric; however, biological support for blood AUC, rather than peak blood concentration, comes from: (1) mechanistic information on RDX binding at the picrotoxin convulsant site of the gamma-amino butyric acid (GABA) channel; and (2) observations from animals studies of convulsions occurring only after repeated exposures. There is evidence from examination of picrotoxin binding to $GABA_A$ that a resulting period of elevated neuronal activity post-exposure could result in increased likelihood of seizures developing over time or other longer-term effects on normal brain function (see Section 1.2.1 for further discussion). Also, as discussed in Section 1.2.1, the range of time to onset of the first observed convulsion in the Crouse et al. (2006) 90-day study was as early as day 10 to as late as day 87, indicating a possible cumulative component of RDX neurotoxicity not accounted for in the currently available mechanistic studies. Therefore, the AUC for RDX concentration in arterial blood was selected as the internal dose metric for analyzing dose-response data for convulsions. Tissue-specific dose metrics for kidney, bladder, and prostate were not available in the PBPK model. Because effects in these organs were observed only after subchronic or chronic exposure to RDX (i.e., there is no evidence that effects are associated with peak exposure) and because greater confidence was placed in model estimates of blood AUC, AUC for RDX concentration in arterial blood was also selected as the internal dose metric for analyzing dose-response data for the kidney, urinary bladder, and prostate. POD_{HED} values based on both blood AUC and peak blood concentration (C_{max}) are presented in Table

2-2 for completeness. As demonstrated in Table 2-2, the POD_{HED}s derived using administered dose, AUC, and C_{max} do not differ greatly; thus, the selection of AUC is not a major determinant of the POD.

The rodent PBPK model was applied to the BMDLs generated from BMD modeling to determine the animal internal dose, expressed as the AUC of RDX blood concentration, and representing the cross-species toxicologically equivalent (internal) dose. The human PBPK model was then applied to derive the corresponding HEDs (see Figure 2-1). Because the AUC is linear with exposure level, at least in the exposure range of interest, the value of the HED would be the same whether the rat or mouse PBPK model is applied before or after BMD modeling is performed. Because the sequence of the calculation does not influence the results, applying the PBPK model after BMD modeling is more efficient—BMD modeling would not have to be redone if there were changes to the PBPK model, and it is easier to evaluate and show two dose metrics (as discussed above). Because of relatively high confidence in the PBPK models developed for the rat and human, these models were used to derive reliable internal dose metrics for extrapolation. For datasets selected from the rat bioassays, the candidate oral values were calculated assuming cross-species toxicological equivalence of the AUC of RDX blood concentration derived from PBPK modeling.

Extrapolation Using $BW^{3/4}$ Scaling.

HEDs were also calculated using a $BW^{3/4}$ scaling approach consistent with EPA guidance (U.S. EPA, 2011). PODs (BMDLs or NOAELs) based on the RDX dose administered in the experimental animal study were adjusted by a standard dosimetric adjustment factor (DAF) derived as follows:

$$DAF = (BW_a^{1/4}/BW_h^{1/4}),$$

where

BW_a = animal body weight

BW_h = human body weight

Using BW_a values of 0.25 kg for rats and 0.036 kg for mice and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs for rats and mice are 0.24 and 0.15, respectively. Applying the DAF to the POD identified for effects in adult rats or mice yields a POD_{HED} as follows (see Table 2-2):

$$POD_{HED} = \text{laboratory animal dose (mg/kg-day)} \times DAF$$

Further details of the BMDL modeling, BMDS outputs, and graphical results for the best fit model for each dataset included in Table 2-2 can be found in Appendix D, Section D.1. Details of the PBPK model evaluation used for extrapolation from BMDL values can be found in Appendix C, Section C.1.5. Table 2-2 summarizes the results of the BMD modeling and the POD_{HED} for each data set discussed above.

2.1.3. Derivation of Candidate Values

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) (Section 4.4.5), and as described in the Preamble, five possible areas of uncertainty and variability were considered when determining the application of UFs to the PODs presented in Table 2-2. An explanation follows:

An intraspecies uncertainty factor, UF_H, of 10 was applied to all PODs to account for potential differences in toxicokinetics and toxicodynamics in the absence of information on the variability of response in the human population following oral exposure to RDX. The available human pharmacokinetic data are not sufficient to inform human kinetic variability and derive a chemical-specific UF for intraspecies uncertainty.

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to all PODs to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between rodents and humans. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of an uncertainty factor of 3. For datasets from the rat bioassays, a PBPK model was used to convert internal doses in rats to external doses in humans (see rationale in Section 2.1.2—Human Extrapolation). This reduces toxicokinetic uncertainty in extrapolating from the rat to humans, but does not account for interspecies differences due to toxicodynamics. A UF_A of 3 was applied to account for this remaining toxicodynamic and any residual toxicokinetic uncertainty not accounted for by the PBPK model.

A subchronic to chronic uncertainty factor, UF_S, of 1 was applied to all PODs. Where a POD was based on a 2-year bioassay (i.e., for urinary system [kidney/urinary bladder] and prostate), a UF_S was not necessary because the RfD was based on effects associated with chronic exposure. Where a POD was based on studies of subchronic exposure to RDX (i.e., for nervous system effects), EPA considered the application of either a UF_S of 3 or 1. Although EPA guidance recommends a default UF_S of 10 on the assumption that effects in a subchronic study would occur at an approximately 10-fold higher concentration than in a corresponding (but absent) chronic study (U.S. EPA, 2002), the RDX database does not support a UF_S of 10. This determination is based on the MOA for nervous system effects and the support across studies that nervous system effects are more strongly driven by dose than duration of exposure (see Section 1.2.1). The argument for the application of a UF_S of 3 is based on some remaining uncertainty regarding the potential for effects to accumulate over time. While the MOA strongly suggests that the convulsive effects of RDX are driven by the transient binding of RDX to target (GABA) receptors in the brain, the lack of complete reversibility of the inhibited GABAergic signaling after removal of RDX in vitro by Williams et al. (2011), as well as observations from related chemicals suggesting that prolonged decreases in inhibitory tone might predispose nervous system tissues to future seizurogenic events (see Section 1.2.1), introduce the possibility that mechanisms leading to cumulative effects over time have not been adequately investigated. The application of a UF_S of 1 is supported by the findings across most studies that convulsions occurred shortly after dosing (minutes to hours) and generally did not

appear to be appreciably influenced by duration of exposure. For example, convulsive effects were observed after a single RDX dose of 12.5 mg/kg-day (the lowest dose tested in this experiment), and several animals exhibited reduced seizure thresholds to other convulsants at 10 mg/kg-day (again, the lowest dose tested) (Burdette et al., 1988). These doses are comparable to the LOAEL of 8 mg/kg-day from the 90-day study by Crouse et al. (2006). Similarly, at 12 and 15 mg/kg-day in the 90-day study by Crouse et al. (2006), neurotoxic signs were present in >80% of animals beginning on day 0 and continuing for the duration of the experiment. Convulsions in Crouse et al. (2006) were not observed until 7 to 15 days of exposure at doses of 10–15 mg/kg-day, and only after 48 days of exposure in rats receiving 8 mg/kg-day RDX (Johnson, 2015a). In a 14-day range-finding study in 6 animals/group, Crouse et al. (2006) reported that neuromuscular signs (tremors, convulsions) were observed at 17 mg/kg-day and above (the next lower dose was 8.5 mg/kg-day). Thus, studies with comparable dosing methods reported seizure-related effects within the narrow range of 8–17 mg/kg-day, regardless of exposure duration (acute to subchronic). Data from chronic rodent studies (Lish et al., 1984; Levine et al., 1983) leads to identification of a higher effective dose range (>35 mg/kg-day) for convulsions than these acute and subchronic exposure studies (and would result in the identification of a higher POD). However, because of cross-study differences in methods of outcome measurement, peak internal dose from gavage administration versus dietary administration, physical form of RDX (e.g., particle size), and dose matrix in the dietary versus gavage preparations that could have influenced absorption rate and internal (e.g., peak) RDX dose, direct comparison of the effective convulsive doses from the available subchronic and chronic studies is not appropriate. Overall, evaluation of the available evidence leaves open the possibility for a small influence of chronic (as compared to subchronic) RDX exposure duration on the manifestation of neurotoxicity; however, current data suggest that any such influence specifically on convulsions would be small and as such a UF_S of 3 would not be warranted. Based on the strong evidence supporting a negligible-to-minimal impact of exposure duration on the effective dose for convulsions, a UF_S of 1 was applied to PODs for neurotoxic effects derived from studies of less-than-chronic duration.

A LOAEL to NOAEL uncertainty factor, UF_L, of 1 was applied to all POD values because every POD was a BMDL or a NOAEL. When the POD is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, the BMR for modeled endpoints was selected under the assumption that the BMR represents a minimal, biologically significant change for these effects.

A database uncertainty factor, UF_D, of 10 was applied to all POD values. The oral toxicity database for RDX includes subchronic and chronic toxicity studies in the rat and mouse, a two-generation reproductive toxicity study in the rat, developmental toxicity studies in the rat and rabbit, and subchronic studies (with study design limitations) in the dog and monkey. As discussed below, some uncertainty is associated with characterization of the RDX neurotoxicity.

EPA prefers to identify reference values based on upstream (less severe) effects that would precede frank effects like convulsions, and uncertainty remains in the understanding of RDX-induced neurotoxicity. In part, this is due to limitations in study design to assess neurotoxicity across the RDX database; the frequency of animal observations in the available studies raises concerns that there may be underreporting of the true incidence of convulsions, and in general the reporting of this effect does not include a measure of the severity at the time of observation. No follow-up studies were identified that employed more sensitive assays to assess more subtle neurotoxicity, and the database lacks a chronic duration study that could inform residual uncertainty regarding the potential for chronic exposure to magnify effects (as compared to subchronic exposure). As noted by the SAB, the convulsion endpoint in rodents does not capture the breadth of potential human hazard, and the lack of information on more sensitive endpoints, including cognitive and behavioral effects, as well as developmental neurotoxicity, is a significant data gap. Uncertainties in the database for RDX neurotoxicity could be addressed by:

- Analysis of "seizures" using more detailed behavioral scoring methods. In the available studies, "convulsion" or "seizure (depending on the reporting in the study) might indicate a range of observable behaviors in response to altered brain activity, ranging from involuntary limb and facial twitches to tonic-clonic seizures in which animals exhibit a sustained (seconds to hours) and widespread loss of muscle control sometimes resulting in respiratory arrest and/or death. As there are studies where convulsions occur at the same dose as mortality, the convulsive activity in these studies is interpreted as severe. Scoring methods quantifying the occurrence of different behavioral aspects of the RDX-induced convulsions, such as the Racine scale (Racine, 1972), employed in Burdette et al. (1988) would provide a much more accurate, complete, and likely more sensitive measure of RDX neurotoxicity.

- Additional electrophysiological measures of epileptiform activity. Well-established and sensitive methods for evaluating brain activity exist. These measures could not only better describe the profile of RDX-induced convulsant activity, but could also be used to identify and quantify sub-convulsive effects of RDX exposure (e.g., EEG spiking). Electrophysiological characterization of the effects of RDX in vitro and in vivo has already been demonstrated by Williams et al. (2011). Additional studies building on this work, looking at the effects of different concentrations of RDX, could potentially identify more sensitive measures of RDX neurotoxicity.

- A FOB conducted by Crouse et al. (2006) provides some limited information on neurobehavioral effects associated with RDX exposure, yet the results of that study did not identify notable effects associated with RDX exposure. While some components of the FOB testing conducted by Crouse et al. (2006) would be expected to give a screening-level evaluation of some stimuli-induced behaviors that have the potential to be related to seizures (e.g., response to handling, touch, click or open field), these observational descriptions are insensitive and are expected to have missed potential subconvulsive effects. Additional studies addressing the potential for subconvulsive behaviors resulting from RDX exposure would be informative. For example, Burdette et al. (1988) examined seizure susceptibility in gavaged male Long Evans rats, at doses ≥10 mg/kg; spontaneous

seizures were already observed in this study at 12.5 mg/kg-day. Further evaluation of seizure susceptibility at lower doses and with longer exposure durations, as well as evaluations of potential effects of subconvulsive doses on subtler behaviors that might be related to RDX neurotoxicity (e.g., motor, anxiety or social behaviors; learning and memory tests) may identify additional measures of RDX neurotoxicity.

- Further evaluation of potential developmental neurotoxicity associated with RDX exposure (see Section 1.3.3 for discussion). Models for examining seizure-related behaviors during development exist, mainly involving manipulation and analyses in pre-weanling rodents. Hess-Ruth et al. (2007) reported possible transfer of RDX to offspring during gestation, as well as the presence of RDX in the milk of dams, indicating a potential for lactational transfer of RDX to offspring. Examination of specific developmental neurotoxicity endpoints has not been conducted in studies of RDX toxicity. Well-conducted developmental neurotoxicity studies could further rule out the possibility that RDX exposure during development might result in immediate or delayed seizure activity, or predispose animals to developing seizures as adults, or it could identify other more sensitive indicators of toxicity.

Overall, while the RDX database adequately covers major systemic effects, including reproductive and developmental effects, uncertainties in the adequacy of the database were identified in characterization of the neurotoxicity hazard. There is significant concern that additional studies described above may lead to identification of a more sensitive endpoint or a lower POD. Accordingly, a UF_D of 10 was applied to all derived PODs.

Table 2-3 is a continuation of Table 2-2 and summarizes the application of UFs to each POD_{HED} to derive a candidate value for each data set. The candidate values presented in Table 2-3 are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for RDX.

Table 2-3. Effects and corresponding derivation of candidate values

Endpoint and reference	POD_{HED}[a]	POD type	UF_A	UF_H	UF_L	UF_S	UF_D	Composite UF	Candidate value (mg/kg-d)
Nervous system (rat)									
Incidence of convulsions Crouse et al. (2006)	1.3	$BMDL_{05}$	3	10	1	1	10	300	4.3×10^{-3}
Incidence of convulsions Cholakis et al. (1980)	0.31	$BMDL_{05}$	3	10	1	1	10	300	1.0×10^{-3}
Incidence of convulsions Levine et al. (1983)	3.9	NOAEL	3	10	1	1	10	300	1.3×10^{-2}
Urinary system (kidney and bladder) (rat)									
Kidney: incidence of medullary papillary necrosis Levine et al. (1983)	3.9	NOAEL	3	10	1	1	10	300	1.3×10^{-2}
Urinary bladder: incidence of *hemorrhagic/ suppurative cystitis* Levine et al. (1983)	5.6	$BMDL_{10}$	3	10	1	1	10	300	1.9×10^{-2}
Prostate (rat)									
Incidence of prostate suppurative inflammation Levine et al. (1983)	0.23	$BMDL_{10}$	3	10	1	1	10	300	7.6×10^{-4}

[a]POD_{HED} values based on data from the rat were derived using PBPK modeling, with the HED based on equivalence of internal RDX dose expressed as AUC for RDX concentration in arterial blood (see Section 2.1.2 and discussion of the PBPK models above and in Appendix C, Section C.1.5).

Figure 2-2 presents graphically the candidate values, UFs, and POD_{HED} values, with each bar corresponding to one data set described in Tables 2-2 and 2-3.

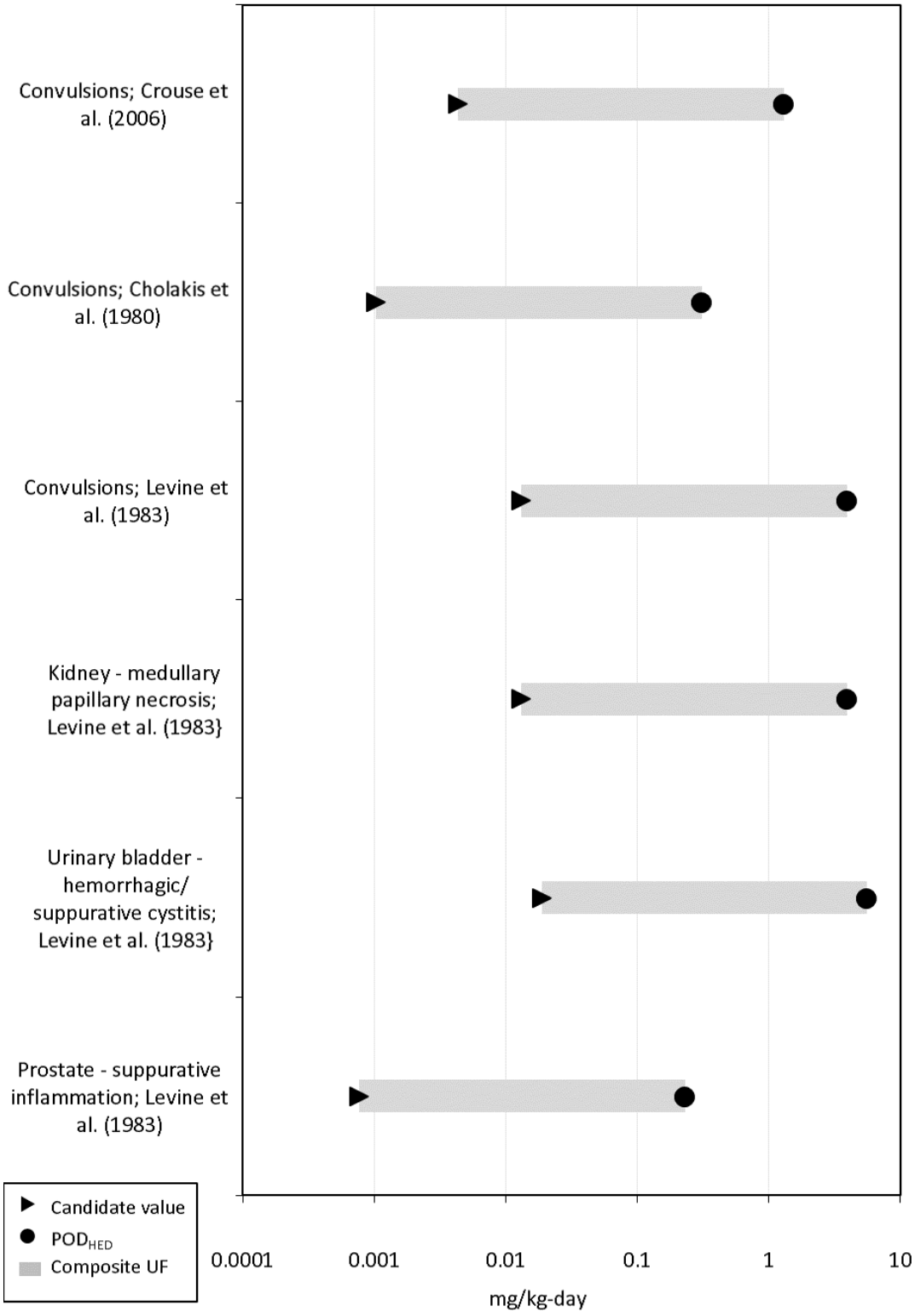


Figure 2-2. Candidate values with corresponding POD and composite UF.

2.1.4. Derivation of Organ/System-Specific Reference Doses

Table 2-4 distills the candidate values from Table 2-3 into a single value for each organ or system. Organ- or system-specific reference values may be useful for cumulative risk assessments that consider the combined effect of multiple agents acting at a common site. For example, organ/system-specific reference values can be used to refine the hazard index (HI)[25] as described in EPA's *Risk Assessment Guidance for Superfund* (U.S. EPA, 1989). As noted by U.S. EPA (1989), one limitation of the HI approach is the potential to overestimate effects when this approach is applied to multiple chemicals that induce different types of effects or do not act by the same mode of action. Availability of organ/system-specific references value allows risk assessors to calculate HIs for chemicals acting at a common site (i.e., and thereby more likely to induce similar effects). Further, derivation of a single reference value for a chemical based on one critical effect only fails to address other potential health effects caused by exposure to that chemical that may occur at exposures higher than those associated with the critical effect. Derivation of organ/system-specific values for all health effects with credible evidence addresses this limitation.

Table 2-4. Organ/system-specific RfDs and overall RfD for RDX

Effect	Basis	RfD (mg/kg-d)	Study exposure description	Confidence
Nervous system	Incidence of convulsions (Crouse et al., 2006)	4×10^{-3}	Subchronic	Medium
Urinary system	Incidence of kidney medullary papillary necrosis (Levine et al., 1983)	1×10^{-2}	Chronic	Medium
Prostate	Incidence of suppurative prostatitis (Levine et al., 1983)	8×10^{-4}	Chronic	Low
Overall RfD	**Nervous system**	**4×10^{-3}**	**Subchronic**	**Medium**

Nervous System Effects

The organ/system-specific RfD for nervous system effects was based on the incidence of convulsions in F344 rats reported in Crouse et al. (2006), a well-conducted study that used a 99.99% pure form of RDX, five closely-spaced dose groups that provided a good characterization of the dose-response curve for convulsions, and an endpoint (convulsions) that was replicated across multiple studies. Although the candidate value derived from the developmental toxicity study in F344 rats by Cholakis et al. (1980) is lower (by approximately fourfold), deficiencies in the Cholakis et al. (1980) study resulted in a candidate value with less confidence than the value derived from

[25]The HI is the sum of more than one hazard quotient for multiple substances and/or multiple exposure pathways, with the hazard quotient derived as the ratio of a single chemical exposure level to an RfD (or RfC) for that chemical *Superfund* (U.S. EPA, 1989).

Crouse et al. (2006). Crouse et al. (2006) was better designed to assess the nervous system effects of RDX, whereas Cholakis et al. (1980) was designed as a developmental toxicity study with only routine monitoring of clinical signs (the methods section states that "Dams were monitored daily for toxic signs"). Crouse et al. (2006) used five dose groups (plus the control) that provided good characterization of the dose-response curve for RDX-induced convulsions, whereas Cholakis et al. (1980) used only three dose group (plus the control) with order of magnitude dose spacing, resulting in a less well-defined characterization of the dose-response curve for this endpoint. Lack of uniformity/homogeneity of the dosing preparation in Cholakis et al. (1980) raised concerns about exposure quality and the potential for under- and over-dosing animals. Cholakis et al. (1980) noted difficulty maintaining uniform dosing suspensions; RDX concentrations in the gavage study ranged from 36 to 501% of target concentrations. In contrast, Crouse et al. (2006) used methods to ensure uniform dosing suspensions; actual RDX concentrations varied from 83 to 114% of target concentrations, and the 114% suspension was adjusted to 100% before administration. In light of evidence that nervous system effects are more strongly driven by dose than duration of exposure (see Section 2.1.3), the wide deviations from the target doses in Cholakis et al. (1980) lead to decreased confidence in the quantitative use of this study. Further, Crouse et al. (2006) used a higher purity test material than did Cholakis et al. (1980) (99.99% versus 88.6%, respectively). Finally, the Crouse et al. (2006) study used a longer exposure duration (90 days) than did the Cholakis et al. (1980) study (14 days), and is more representative of a chronic exposure duration. The lower candidate value from the Cholakis et al. (1980) developmental toxicity study could indicate that pregnant animals are a susceptible population, which could support selection of this study as the basis for the RfD; however, as discussed in Section 1.3.3, the available studies in pregnant and nonpregnant rats cannot be directly compared, and the available information is not considered sufficient to identify pregnant animals as a susceptible population.

As discussed in Section 2.1.1, the 2-year dietary study by Levine et al. (1983) was also considered for RfD derivation because the available oral studies suggest that bolus doses of RDX received with gavage administration may induce nervous system effects at doses lower than those resulting from dietary administration (recognizing that differences in particle size and purity of the test material may confound direct comparisons between gavage and dietary administration). Convulsion data from Levine et al. (1983) yielded a POD_{HED} threefold higher than the POD_{HED} derived from Crouse et al. (2006). The POD derived from the Levine et al. (1983) study is considered less certain than that derived from Crouse et al. (2006). Levine et al. (1983) did not provide information on the incidence of neurotoxic effects, and BMD analysis was thus not supported (i.e., the POD was based on a NOAEL). As discussed in Section 1.2.1, the frequency of daily observations in the Levine et al. (1983) study may not have been sufficient to provide an accurate measure of the occurrence of nervous system effects, potentially leading to underestimation of convulsions and other nervous system effects. For these reasons, and in light of

the fact that data from the Levine et al. (1983) study yielded a higher POD, Levine et al. (1983) was not used as the basis for the organ/system-specific RfD for nervous system effects.

Urinary System (Kidney and Bladder) Effects

Dose-response analysis was conducted for two data sets representing effects on the urinary system—incidence of medullary papillary necrosis in the kidney and incidence of hemorrhagic/suppurative cystitis in the urinary bladder, both as reported by Levine et al. (1983). Both effects were reported primarily in high-dose male rats in this study, and both data sets yielded similar POD_{HED}s (3.9 and 5.6 mg/kg-day, respectively) and candidate values (1.3×10^{-2} and 1.9×10^{-2} mg/kg-day, respectively). The smaller of the two candidate values (1.3×10^{-2} mg/kg-day) was selected as the organ/system-specific RfD for urinary system effects.

Prostate Effects

A single data set for prostate effects, specifically the incidence of suppurative prostatitis in male F344 rats as reported in a 2-year dietary study by Levine et al. (1983), was brought forward for quantitative analysis. The organ/system-specific RfD for prostate effects is based on this dataset.

2.1.5. Selection of the Overall Reference Dose

Multiple organ/system-specific reference doses were derived for effects identified as hazards from RDX exposure, including organ/system-specific reference doses for the nervous system, urinary system (kidney and bladder), and prostate. There is strong support for RDX as a nervous system toxicant, with evidence for nervous system effects, and specifically convulsions, observed in humans and in multiple experimental animal studies, in multiple species, and following a range of exposure durations.

The organ/system-specific RfD for nervous system effects of 4×10^{-3} mg/kg-day is smaller than the organ/system-specific RfD for urinary system effects (1×10^{-2} mg/kg-day), suggesting that the RfD for nervous system effects is protective of effects on both organ systems. This is consistent with findings from animal bioassays that show RDX-related effects on the kidney and urinary bladder at exposure levels higher than those associated with convulsions.

The organ/system-specific RfD for nervous system effects is fivefold higher than the organ/system-specific RfD for prostate effects of 8×10^{-4} mg/kg-day. Although smaller in value, the RfD for prostate effects was not selected as the overall RfD. Evidence for dose-related effects on the prostate comes from a single 2-year toxicity study in male rats (Levine et al., 1983); a second chronic study in the rat that evaluated prostate histopathology was not available, and the 2-year study in mice (Lish et al., 1984) did not identify similar patterns of prostate inflammation. There are also uncertainties in the diagnosis of suppurative prostatitis. Levine et al. (1983) do not provide more extensive detail on the histopathological evaluation of the prostate to account for potential variation in inflammation inherent to the different lobes of the prostate. Additionally,

male rats in the high-dose group (40 mg/kg-day) were moved from group to individual housing during weeks 30–40 on study, due to the high incidence of fighting. As noted by the SAB, Creasy et al. (2012) reported that fighting may cause urogenital infections in male rats. The fighting observed by Levine et al. (1983), along with the change in housing conditions from the other treatment groups, increases uncertainty in the response of the high-dose group.

Therefore, the organ/system-specific RfD of **4×10^{-3} mg/kg-day** for nervous system effects in the rat as reported by Crouse et al. (2006) is selected as the overall RfD for RDX given the strength of evidence for the nervous system as a hazard of RDX exposure, and the greater confidence in the value for nervous system effects compared to urinary system and prostate effects.

The overall RfD is derived to be protective of all types of effects for a given duration of exposure, and is intended to protect the population as a whole, including potentially susceptible subgroups (U.S. EPA, 2002). Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. In the case of RDX, no specific lifestages have been identified as a potentially susceptible subgroup.

2.1.6. Comparison with Mortality LD_{01}s

As previously discussed, mortality was considered in discussions of other organ/system-specific toxicity (and in particular, effects on the nervous system and kidney). EPA did not develop an RfD for mortality because EPA generally does not develop reference values based on frank effects such as mortality; rather, reference values are generally based on earlier (less severe) upstream events, where possible, in order to protect against all adverse outcomes. Nevertheless, additional analysis of mortality data was undertaken because some studies (see Table 2-5) identified mortality at the same RDX dose that induced nervous system effects (Crouse et al. (2006); Angerhofer et al. (1986); Cholakis et al. (1980); von Oettingen et al. (1949)).

Table 2-5. Comparison of dose levels associated with mortality and convulsions in selected studies

Study	Doses associated with mortality	Doses associated with convulsions
Crouse et al. (2006) Rats, F344, 10/sex/group 0, 4, 8, 10, 12, or 15 mg/kg-d 13 wks/gavage	≥8 mg/kg-d	≥8 mg/kg-d
von Oettingen et al. (1949) Rats, sex/strain not specified, 20/group 0, 15, 25, or 50 mg/kg-d 13 wks/diet	≥25 mg/kg-day	≥25 mg/kg-d
Cholakis et al. (1980) Rats, F344, 24–25 females/group 0, 0.2, 2.0, or 20 mg/kg-d GDs 6–19/gavage	20 mg/kg-d	Primarily 20 mg/kg-d; 1 convulsion at 2 mg/kg-d
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group 0, 2, 6, or 20 mg/kg-d GDs 6–15/gavage	Primarily at 20 mg/kg-d, but one death each at 2 and 6 mg/kg-d	20 mg/kg-d

A discussion of mortality evidence for RDX is presented in Appendix C, Section C.3.1, and the relationship between mortality and nervous system effects in Sections 1.2.1 and 1.3.1. Unscheduled deaths were observed as early as day 8 of a 90-day gavage study (Crouse et al., 2006) and in developmental toxicity studies with exposure durations of two weeks (Angerhofer et al. (1986); Cholakis et al. (1980)).

Given the proximity in the dose at which mortality and nervous system effects were observed in several studies, the dose-response relationships for mortality were compared across studies with durations similar to those in Table 2-5 by comparing the LD_{01} (the dose expected to be lethal to 1% of the animals) or NOAELs derived from each study. A BMR of 1% ER was used for modeling mortality data in light of the severity of this frank effect. In addition, the LD_{01} values and NOAELs for mortality were compared to BMD_{01} for convulsions.[26] For purposes of this analysis, a BMR of 1% ER was selected for convulsions (rather than 5% ER used in the analysis to derive the nervous system RfD) to facilitate comparison with the LD_{01} values for mortality.

Interpretation of mortality data from chronic exposure studies in mice and rats is complicated by other treatment-related effects and pathology regularly observed in aging animals

[26]BMDs were compared, as opposed to BMDLs, because, as stated on p. 20 of the BMD Technical Guidance (U.S. EPA, 2012a), "In general, it is recommended that comparisons across chemicals/studies/endpoints be based on central estimates; this is in contrast to using lower bounds for PODs for reference values..."

(e.g., kidney pathology, neoplastic lesions), and was not considered in this analysis. Other studies that were less informative and not considered in this analysis are not presented in Table 2-6.[27]

Table 2-6. Summary of dose-response evaluation for mortality following oral exposure to RDX

Reference (exposure duration/route)	Species/sex	Model[a]	BMR	LD_{01} (mg/kg-d)	LDL_{01} (mg/kg-d)
Diet studies					
Lish et al. (1984) (11-wk data from 2-yr study/diet)	Male and female $B6C3F_1$ mouse	Not amenable to modeling	NOAEL: 35 mg/kg-d 95% CI for response: 0–4%		
Levine et al. (1981a) (13-wk/diet)	Male and female F344 rat, combined	Multistage 4[o]	1% ER	7.9	2.2
von Oettingen et al. (1949) (13-wk/diet)	Rats, sex/strain not specified	Not amenable to modeling	NOAEL: 15 mg/kg-d 95% CI for response: 0–15%		
Cholakis et al. (1980) (2-generation design/diet)	Female CD rat	Not amenable to modeling	NOAEL: 16 mg/kg-d 95% CI for response: 0–13%		
Levine et al. (1983) (13-week data from 2-yr study/diet)	Male and female F344 rat	NA (no mortality at highest dose tested)	NOAEL: 40 mg/kg-d 95% CI for response: 0–4%		
Cholakis et al. (1980) (13-wk/diet)	Male and female F344 rat	NA (no mortality at highest dose tested)	NOAEL: 40 mg/kg-d 95% CI for response: 0–25%		

[27]The following less informative studies were not included in the analysis of early mortality:
13-week dietary study in the mouse by Cholakis et al. (1980). Mortality was observed only in the high-dose group (257–276 mg/kg-day TWA), and the unusual dosing regimen precluded identification of a NOAEL or LOAEL.
13-week dietary study in the dog by Hart (1974) and 13-week study in the monkey by Martin and Hart (1974). Both studies used small group sizes (3 animals/dose group), and no animals died on study (although one high-dose monkey was euthanized).
6-week dietary study in the dog from the 1949 publication by von Oettingen et al. (1949). This dog study included only one treatment group and recorded only one death.
30-day gavage study in the rat by MacPhail et al. (1985). The authors did not identify treatment-related mortality, but reporting was limited.

Reference (exposure duration/route)	Species/sex	Model[a]	BMR	LD_{01} (mg/kg-d)	LDL_{01} (mg/kg-d)
Gavage studies					
Crouse et al. (2006) (90-d/gavage)	Male and female F344 rat, combined	Multistage 2^o	1% ER	2.1	0.46
Cholakis et al. (1980) (GDs 6–19/gavage)	Female F344 rat	Not amenable to modeling	NOAEL: 2 mg/kg-day 95% CI for response: 0–12%		
Angerhofer et al. (1986) (GD 6–15/gavage)	Female SD rat	Multistage 3^o	1% ER	1.7	0.59
Cholakis et al. (1980) (GDs 7–29/gavage)	Female New Zealand white rabbit	NA (no mortality at highest dose tested)	NOAEL: 20 mg/kg-day 95% CI for response: 0–22%		

[a]For modeling details, see Appendix D, Section D.1.2, Tables D-9 to D-12.

CI = confidence interval; ER = extra risk; LD_{01} = dose expected to be lethal to 1% of the animals; LDL_{01} = lower confidence limit on the LD_{01}.

Of the studies in Table 2-6, dose-response analysis was conducted for all studies that showed an increased incidence of unscheduled deaths. LD_{01} values are provided in Table 2-6, and detailed modeling results are provided in Appendix D, Section D.1.2. Mortality was observed only at the highest dose tested at week 11 in the 2-year mouse study by Lish et al. (1984), in the 13-week rat study by von Oettingen et al. (1949), and in the two-generation reproductive and developmental toxicity studies by Cholakis et al. (1980). In these cases, data were not amenable to LD_{01} estimation, and a NOAEL (with a confidence interval, CI, on its associated response) was used in this comparative analysis instead.

LD_{01} values for mortality in Table 2-6 range from 1.7 mg/kg-day (10-day gavage exposure in pregnant rats) to 7.9 mg/kg-day (13-week dietary exposure in rats), with the lower values generally from studies that administered RDX by gavage. These values may be compared to the BMD_{01} for convulsions from Crouse et al. (2006) (see Appendix D, Table D-3). The BMD_{01} for convulsions of 3.0 mg/kg-day is in the middle of the distribution of calculated LD_{01}s, and the lowest LD_{01} of 1.7 mg/kg-day is within twofold of the convulsion BMD_{01} of 3.0 mg/kg-day.

The NOAELs from studies where mortality was observed tend to be higher than the LD_{01}s. However, NOAELs are not directly comparable to BMD_{01}s for several reasons. CIs for the responses characterize some statistical uncertainty for NOAELs from studies that could not be modeled (note that the upper bound of a CI is not directly comparable to a lower bound on a benchmark dose). The CIs suggest that comparable 1% levels for these datasets could be lower than the NOAELs. In addition, dose-spacing can affect the interpretation of NOAELs, such as that from the Cholakis et al. (1980) developmental toxicity study because of the wide (order-of-magnitude) spacing between doses in that study (i.e., the reported NOAEL of 2 mg/kg-day [see Table 2-6] is 10-fold lower than

the dose associated with 21% mortality (5/24 dams) at 20 mg/kg-day [see Appendix C, Table C-10]).

In general, this comparison indicates that PODs derived from mortality data would be similar to PODs for RDX based on convulsions. The proximity of doses associated with mortality and nervous system effects should be taken into consideration when assessing health risks from environmental exposures to RDX.

2.1.7. Uncertainties in the Derivation of the Reference Dose

To derive the RfD, the UF approach (U.S. EPA, 2000, 1994) was applied to a POD_{HED} based on nervous system effects in rats exposed to RDX for a subchronic duration. UFs were applied to the POD_{HED} values to account for uncertainties in extrapolating from an animal bioassay to human exposure, the likely existence of a diverse human population of varying susceptibilities, and to address limitations in the database. For the most part, these extrapolations are carried out with default approaches given the lack of data to inform individual steps. One exception is the use of PBPK modeling to perform interspecies (i.e., rat to human) extrapolation. Uncertainties associated with the PBPK models are considered in Appendix C, Section C.1.5.

Nervous system effects have been documented in multiple studies and animal species and strains; however, some uncertainty is associated with the incidence of reported neurological effects in studies that employed a study design that did not monitor animals with sufficient frequency to accurately record neurobehavioral effects, including convulsions. In the study used to derive the RfD (Crouse et al., 2006), Johnson (2015a) noted that convulsions were observed infrequently outside the dosing period; more often, seizures were observed during the 2-hour (gavage) dosing period, typically within 60–90 minutes of dosing. Similar information was not available for other studies to assess the likelihood that observations of convulsions were missed. However, animals were not monitored continuously during the Crouse et al. (2006) study, and investigators reported that nearly all observed pre-term deaths in rats exposed to the three higher doses were preceded by signs of neurotoxicity. If an animal died during the study as a result of effects on the nervous system, convulsions preceding death could have been missed, resulting in an underestimation of the incidence of convulsions. Conversely, attributing all mortality to neurotoxicity (i.e., all deaths were preceded by convulsions that may not have been observed) could result in an overestimation of the incidence of convulsions. A dose-response analysis of the combined incidence of seizures and mortality from Crouse et al. (2006) was conducted to evaluate the impact of these assumptions, as the true convulsion incidence would likely fall somewhere between the observed convulsion incidence and the combined incidence of convulsions and mortality. This analysis revealed that the POD_{HED} of 0.24 mg/kg-day for a combined incidence of convulsions and mortality[28] was similar to

[28]The POD_{HED} values were derived from data in Crouse et al. (2006) using a BMR of 1% ER and PBPK modeling (see Section 2.1.2 and discussion of the PBPK models in Appendix C, Section C.1.5).

the POD_{HED} of 0.28 mg/kg-day for convulsions alone (using a BMR of 1% ER for comparability to the analysis with mortality data), indicating that potential underestimation of convulsion incidence in the Crouse et al. (2006) study was not likely to impact the RfD.

Some uncertainty is also associated with the influence of the method of oral dosing on the magnitude of dose required to induce nervous system effects. As noted in Section 1.2.1, gavage administration generally induced convulsions in experimental animals at lower doses than did dietary administration, possibly due to the bolus dose resulting from gavage administration that could lead to comparatively faster absorption and higher peak blood concentrations of RDX. To some extent, this uncertainty is reflected in the threefold difference in the candidate POD_{HED} values derived from the Crouse et al. (2006) (gavage administration) and Levine et al. (1983) (dietary administration) studies. A more rigorous examination of the effect of oral dosing method cannot be performed because of the differences in test materials and study designs used in the available gavage and dietary studies that could also have contributed to differences in response (e.g., test article purity and particle size, number and spacing of dose groups, exposure duration, frequency of clinical observations, and thoroughness of the reporting of observations).

Other sources of uncertainty related to the RDX database have already been discussed at length, namely the lack of more sensitive measures of neurotoxicity than convulsions, the lack of studies examining the potential for RDX exposure to cause developmental neurotoxicity, and the possibility for some increase in the incidence of neurotoxic effects with cumulative exposure (see Sections 1.3.3 and 2.1.3). The use of a BMR of 5% addresses some of the concern for quantification of a frank effect such as convulsions, while application of a UF_D of 10 addresses limitations in the sensitivity of the neurotoxicity measures as well as the lack of a developmental neurotoxicity study.

2.1.8. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfD is medium. Confidence in the principal study (Crouse et al., 2006) is high. The study was well-conducted, utilized 99.99% pure RDX, and had five closely-spaced dose groups that allowed characterization of dose-response curves for convulsions in the dose range of interest. One limitation identified by study authors was the limited ability of the FOB to fully identify neurobehavioral effects at doses ≥8 mg/kg-day due to the timing of the dosing procedure and timing of the FOB screening. Confidence in the database is medium to low. The database includes three chronic studies in rats and mice; eight subchronic studies in rats, mice, dogs, and monkeys; two short-term studies; and four reproductive/developmental toxicity studies

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Calculation of $POD_{HED\text{-}01}$ based on incidence of convulsions: $BMDL_{01}$ = 0.569 mg/kg-day (see Appendix D.1.2, Table D-3); converted to $POD_{HED\text{-}01}$ based on AUC for RDX in arterial blood = 0.28 mg/kg-day.
Calculation of $POD_{HED\text{-}01}$ based on incidence of convulsions and mortality: $BMDL_{01}$ = 0.49 mg/kg-day (see Appendix D.1.2, Table D-5); converted to $POD_{HED\text{-}01}$ based on AUC for RDX in arterial blood = 0.24 mg/kg-day.

in rats and rabbits (including a two-generation reproductive study). Confidence in the database is reduced largely because of (1) differences in test material used across studies, (2) uncertainties in the influence of oral dosing methods, and (3) limitations in the available studies to fully characterize potential neurological effects and developmental neurotoxicity. As discussed in Section 2.1.7 and Appendix C, Section C.1.5, differences in test material formulation and particle size may affect RDX absorption and subsequent toxicity, which in turn could influence the characterization and integration of toxicity findings across studies. The available evidence also suggests that bolus dosing of RDX that results from gavage administration induces neurotoxicity at doses lower than administration in the diet, although a rigorous examination of these differences cannot be performed with the available database. To the extent that a bolus dose of RDX, with associated high peak blood concentrations, may not represent likely human exposure, the use of toxicity data from a gavage (bolus dosing) study may introduce uncertainty in the RfD. Finally, as noted in Section 1.2.1 and 1.3.3, the convulsion incidence endpoint in rodents does not reflect the spectrum of potential human hazard; the lack of information on developmental neurotoxicity, as well as more sensitive cognitive and behavioral effects, introduces uncertainty into the derived RfD. Reflecting high confidence in the principal study and medium to low confidence in the database, overall confidence in the RfD is medium.

2.1.9. Previous IRIS Assessment

The previous RfD for RDX, posted to the Integrated Risk Information System (IRIS) database in 1988, was based on a 2-year rat feeding study by Levine et al. (1983). The no-observed-effect level (NOEL) of 0.3 mg/kg-day based on suppurative inflammation of the prostate in male F344 rats from this study was identified as the POD. An RfD of 3×10^{-3} mg/kg-day was derived following application of an overall UF of 100 ($UF_A = 10$, $UF_H = 10$).

2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The inhalation reference concentration (RfC, expressed in units of mg/m^3) is defined as 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 effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

As discussed in Section 1.3.1, the available inhalation literature does not support characterization of the health hazards specifically associated with chronic inhalation exposure to RDX, nor do the studies support quantitative dose-response analysis. Of the available human epidemiological studies of RDX (West and Stafford, 1997; Ma and Li, 1993; Hathaway and Buck, 1977), none provided data that could be used for dose-response analysis. The studies by Ma and Li

(1993) of neurobehavioral effects in Chinese workers and West and Stafford (1997) of hematological abnormalities in ordnance factory workers had numerous methodological limitations that preclude their use for quantitative analysis (see Literature Search Strategy | Study Selection and Evaluation). The study by Hathaway and Buck (1977) found no evidence of adverse health effects in munition plant workers (based on evaluation of liver function, renal function, and hematology), and therefore does not identify a POD at which there would be an effect from which to derive an RfC. Multiple case reports provide some evidence of effects in humans associated with acute exposure to RDX; however, while case reports can support the identification of hazards associated with RDX exposure, data from case reports are inadequate for dose-response analysis and subsequent derivation of a chronic reference value because of short exposure durations and incomplete or missing quantitative exposure information.

As discussed in Literature Search Strategy | Study Selection and Evaluation, a single experimental animal study involving inhalation exposure was identified in the Defense Technical Information Center (DTIC) database; the study is not publicly available. However, the study would not have provided useful data on responses to inhaled RDX, as it was limited by small numbers of animals tested, lack of controls, and incomplete reporting of exposure levels.

Therefore, the available health effects literature does not support the derivation of an RfC for RDX. While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation from the RfD.

2.2.1. Previous IRIS Assessment

An RfC for RDX was not previously derived under the IRIS Program.

2.3. ORAL SLOPE FACTOR FOR CANCER

The oral slope factor (OSF) is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. The OSF can be multiplied by an estimate of lifetime exposure (in mg/kg-day) to estimate the lifetime cancer risk.

2.3.1. Analysis of Carcinogenicity Data

As noted in Section 1.3.2, there is "suggestive evidence of carcinogenic potential" for RDX. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state:

> When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities.

In the case of RDX, there are well-conducted studies that tested large numbers of animals at multiple dose levels (Lish et al., 1984; Levine et al., 1983), making the cancer response suitable for dose-response analysis. Considering the data from these studies, along with the uncertainty associated with the suggestive nature of the weight of evidence, quantitative analysis of the tumor data may be useful for providing a sense of the magnitude of potential carcinogenic risk.

The incidences of liver and lung tumors in female mice from the study by Lish et al. (1984) were selected for quantitative dose-response analysis. The study by Lish et al. (1984): (1) included comprehensive histopathological examination of major organs; (2) contained four dose groups and a control; (3) used adequate numbers of animals per dose group (85/sex/group, with interim sacrifice groups of 10/sex/group at 6 and 12 months) and a sufficient overall exposure duration (2 years); and (4) adequately reported methods and results (including individual animal data). Female mouse liver tissues from the original unpublished study by Lish et al. (1984) were reevaluated by a pathology working group (PWG) (Parker et al., 2006) in order to apply more up-to-date histopathological criteria established by Harada et al. (1999). The updated liver tumor incidences from the PWG reanalysis of Lish et al. (1984) were used for quantitative dose-response analysis.

In the case of both liver and lung tumors, benign and malignant tumors (i.e., adenomas and carcinomas) were combined for dose-response analysis because benign and malignant tumors in both organs develop from the same cell line and there is evidence for progression from benign to the malignant stage (U.S. EPA, 2005a; McConnell et al., 1986). In addition, the highest dose group was excluded from the analyses because of the death of almost half the animals in that group from overdosing. As a group, mice that survived exposure to 175 mg/kg-day RDX for 11 weeks may not have constituted an unbiased representation of the population of animals exposed to the final high dose of 100 mg/kg-day from week 11 to study termination at 2 years. These animals may have been more or less sensitive to RDX than the animals in the general population, and there is no way to determine to what degree. Therefore, this group was excluded because its tumor rates may not have been representative of the population tumor rate at this dose. Female mouse liver and lung tumor incidences from the Lish et al. (1984) study are summarized in Appendix D, Table D-13.

The incidence of hepatocellular carcinomas in male F344 rats from the study by Levine et al. (1983) and the incidence of alveolar/bronchiolar carcinomas in male B6C3F$_1$ mice from the study by Lish et al. (1984) were also considered for quantitative dose-response analysis. Both studies were well-conducted, using similar study designs (described above). In both instances, the response was less robust than the response observed in female mice from the Lish et al. (1984) study. The hepatocellular carcinoma result in male F344 rats is based on a small number of tumors (1/55, 0/55, 0/52, 2/55, and 2/31, respectively, at 0, 0.3, 1.5, 8.0, and 40 mg/kg-day), and inferences made from such a sparse response are uncertain. There was no increased trend in hepatocellular adenomas and carcinomas combined. The alveolar/bronchiolar carcinomas in male B6C3F$_1$ mice showed a positive trend; however, a positive trend was not observed when the

incidence of adenomas and carcinomas was combined. Modeling results are provided in Appendix D, Section D.2.3 for comparison.

2.3.2. Dose-Response Analysis—Adjustments and Extrapolation Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical be determined by what is known about the mode of action (MOA) of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended when there are MOA data to indicate that the dose-response curve is expected to have a linear component below the POD or when the weight-of-evidence evaluation of all available data are insufficient to establish the MOA for a tumor site (U.S. EPA, 2005a). In the case of RDX, the mode of carcinogenic action for hepatocellular and alveolar/bronchiolar tumors is unknown (see discussion of Mechanistic Evidence in Section 1.2.7). Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with RDX exposure.

The survival curves were compared across dose groups in each study to determine whether time of death should be incorporated in the dose-response analysis of tumors. For female mice in Lish et al. (1984), the survival curves were determined to be similar across dose groups after excluding the high-dose group (log-rank test, p-value ≥0.10); therefore, a time-to-tumor analysis was not necessary for this study. Tumor incidence was modeled using the multistage-cancer models in BMDS (versions 2.4 and 2.5). A standard BMR of 10% ER was applied to both tumor sites in the mouse.

Given the finding of an association between RDX exposure in the female mouse and increased tumor incidence at two tumor sites, basing the OSF on only one tumor site could potentially underestimate the carcinogenic potential of RDX. Therefore, an analysis that combines the results from the mouse liver and lung tumor incidence is preferred. The MS-COMBO procedure (BMDS, version 2.5) extends the multistage-cancer models to the case with multiple tumors assuming independence between tumor types. There is no known biological relationship between liver and lung tumors in RDX-exposed mice, and therefore, as noted by the National Research Council (NRC, 1994), this assumption of independence is not considered likely to produce substantial error in risk estimates. The procedure derives a maximum likelihood estimate of the combined risk at a 95% confidence level based on the parameter values obtained for the individual tumor multistage model fits. Additional details on the MS-COMBO procedure are provided in Appendix D, Section D.2.1.

In addition, a sensitivity analysis was conducted as recommended by the SAB in their evaluation of the external review draft of the RDX assessment (SAB, 2017). The SAB recommended this analysis to investigate 1) the fit of the multistage models in the low-dose region; 2) the effect of dropping the highest dose group; and 3) the impact of low concurrent controls on model selection and the POD estimate. The sensitivity analysis is provided in Appendix D, Section D.2.4.

EPA's preferred approach for extrapolating results from animal studies to humans is toxicokinetic modeling. As described in Appendix C, Section C.1.5, PBPK models for RDX in mice and humans published by Sweeney et al. (2012b) were evaluated and further developed by EPA. Consideration was given to whether the available toxicokinetic information supported using an internal dose metric derived by PBPK modeling. The available mechanistic data (Section 1.2.7) point to some evidence, although not conclusive, that RDX-generated metabolites may be implicated in the observed tumorigenicity in the female mouse. However, there are no data on the toxicokinetics of RDX metabolites, and metabolism in the liver is the only route of elimination of RDX in the PBPK model. In this case, as is to be expected from mass balance principles, the PBPK modeling provides no further information; the HED obtained from the model-estimated amount of total RDX metabolites scaled by $BW^{3/4}$ was equal to that calculated using administered dose scaled by $BW^{3/4}$.

In addition to the lack of data on metabolism, other major uncertainties were identified in the mouse PBPK modeling. The mouse model was based on fitting both the absorption and metabolic rate constants to a single set of blood concentration measurements. In this study, the lowest dose that resulted in a detectable level of RDX in blood was 35 mg/kg, a dose high enough to manifest some toxicity in the chronic mouse bioassay. At the 4-hour timepoint in this study, measurement of blood RDX was based on results from only one of six exposed mice (the five other data points were non-detects, excluded as an outlier, or not collected because of death) (Sweeney et al., 2012b). The type of additional data that increased confidence in the rat and human models (e.g., in vitro measurements of RDX metabolism and RDX elimination data) are not available for mice. Consequently, confidence in the mouse model parameter values and in the calibration of the mouse PBPK model is low. Further, there are no data to enable characterizing the fraction of RDX that is metabolized in the mouse; this is problematic considering evidence that indicates that the role of metabolism in RDX toxicity may differ across species (e.g., mice may have more efficient or higher expression of the cytochrome P450 [CYP450] enzymes). Given the high sensitivity of the model to the metabolic rate constant, the uncertainty in mouse toxicokinetics significantly decreases confidence in using the mouse PBPK model for predicting mouse blood RDX concentrations. (See Summary of Confidence in PBPK Models for RDX in Appendix C, Section C.1.5 for further discussion of confidence in the mouse model.) In light of insufficient toxicokinetic information to identify a supported internal dose metric and model uncertainties, the PBPK model developed for the mouse was not used. Consistent with the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the approach used to calculate an HED from the mouse tumors, in the absence of a suitable PBPK model, was adjustment of the administered dose by allometric scaling to achieve toxicological equivalence across species.

As discussed in Section 2.1.1, the administered dose in animals was converted to an HED on the basis of $(\text{body weight})^{3/4}$ (U.S. EPA, 1992). This was accomplished by multiplying administered dose by $(\text{animal body weight in kg/human body weight in kg})^{1/4}$ (U.S. EPA, 1992), where the body

weight for the mouse is 0.036 kg and the reference body weight for humans is 70 kg (U.S. EPA, 1988). Details of the BMD modeling can be found in Appendix D, Section D.2.

2.3.3. Derivation of the Oral Slope Factor

The lifetime cancer OSF for humans is defined as the slope of the line from the BMR (10% ER) at the BMDL to the estimated control response at zero (OSF = 0.1/$BMDL_{10\text{-HED}}$). This slope, a 95% upper confidence limit on the true slope, represents a plausible upper bound on the true slope or risk per unit dose. The PODs estimated for each mouse tumor site are summarized in Table 2-7. Using linear extrapolation from the $BMDL_{10\text{-HED}}$, human equivalent OSFs were derived for each tumor site individually and both sites combined and are listed in Table 2-7.

Table 2-7. Model predictions and OSFs for hepatocellular and alveolar/bronchiolar adenomas or carcinomas in female $B6C3F_1$ mice administered RDX in the diet for 2 years (Lish et al., 1984)

Tumor type	Selected model[a]	BMR	BMD (mg/kg-d)	BMDL (mg/kg-d)	$BMD_{10\text{-HED}}$[b] (mg/kg-d)	POD = $BMDL_{10\text{-HED}}$[c] (mg/kg-d)	OSF[d] $(mg/kg\text{-}d)^{-1}$
Hepatocellular adenomas or carcinomas[e]	Multistage 1°	10% ER	25.5	14.2	3.81	2.12	0.047
Alveolar/ bronchiolar adenomas or carcinomas	Multistage 1°	10% ER	29.9	14.9	4.47	2.23	0.045
Liver + lung tumors	Multistage 1° (MS-COMBO)	10% ER	13.8[f]	8.53[f]	2.06	1.28	0.078

[a]The highest dose was dropped prior to analysis (see Section 2.3.1).
[b]$BMD_{10\text{-HED}} = BMD_{10} \times (BW_a^{1/4}/BW_h^{1/4})$, where BW_a = 0.036 kg, and BW_h = 70 kg.
[c]$BMDL_{10\text{-HED}} = BMDL_{10} \times (BW_a^{1/4}/BW_h^{1/4})$, where BW_a = 0.036 kg, and BW_h = 70 kg.
[d]OSF = $BMR/BMDL_{10\text{-HED}}$, where BMR = 0.1 (10% ER).
[e]Incidences of female mouse liver tumors from Lish et al. (1984) are those reported in the PWG reevaluation (Parker et al., 2006).
[f]Data for hepatocellular adenomas and carcinomas and for liver and lung tumors combined were remodeled using the original sample sizes provided in Lish et al. (1984), which were slightly different for two groups than those reported in Parker et al. (2006). The resulting BMDs and BMDLs from the remodeling were 25.7 and 14.3 mg/kg-day, respectively, for hepatocellular adenomas and carcinomas and 13.8 and 8.56 mg/kg-day, respectively, for liver and lung tumors combined. See Appendix D, Table D-16 and the subsequent MS-COMBO results for details.

An OSF was derived from the $BMDL_{10\text{-HED}}$ based on a significantly increased trend in the incidence of hepatocellular and alveolar/bronchiolar adenomas or carcinomas in female $B6C3F_1$ mice (i.e., the Liver + Lung $BMDL_{10\text{-HED}}$ from MS-COMBO). The OSF of **0.08 $(mg/kg\text{-}day)^{-1}$** is

calculated by dividing the BMR (10% ER) by the Liver + Lung $BMDL_{10\text{-HED}}$ and represents an upper bound on cancer risk per unit dose associated with a continuous lifetime exposure:

$$\text{OSF} = 0.10 \div \text{(Liver + Lung) } BMDL_{10\text{-HED}} = 0.10 \div 1.28 \text{ mg/kg-day}$$

$$= 7.8 \times 10^{-2} \text{ (mg/kg-day)}^{-1}$$

$$= 8 \times 10^{-2} \text{ (mg/kg-day)}^{-1}\text{, rounded to one significant figure}$$

The slope of the linear extrapolation from the central estimate of exposure associated with 10% extra cancer risk ($BMD_{10\text{-HED}}$) from the same data sets is given by:

Slope of the linear extrapolation from the central estimate

$$= 0.10 \div \text{(Liver + Lung) } BMD_{10\text{-HED}} = 0.10 \div 2.06 \text{ mg/kg-day}$$

$$= 4.9 \times 10^{-2} \text{ (mg/kg-day)}^{-1}$$

$$= 5 \times 10^{-2} \text{ (mg/kg-day)}^{-1} \text{ (rounded to one significant figure)}$$

The OSF for RDX should not be used with exposures exceeding the POD (1.28 mg/kg-day), because above this level, the fitted dose-response model better characterizes what is known about the carcinogenicity of RDX.

2.3.4. Uncertainties in the Derivation of the Oral Slope Factor

A number of uncertainties underlie the cancer unit risk for RDX. Table 2-8 summarizes the impact on the assessment of issues such as the use of models and extrapolation approaches particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the effect of reasonable alternatives, the approach selected, and its justification.

Table 2-8. Summary of uncertainty in the derivation of the cancer risk value for RDX

Consideration and impact on cancer risk value	Decision	Justification
Selection of study The cancer bioassay in the rat (Levine et al., 1983) would provide a lower estimate of the OSF	Lish et al. (1984) as principal oral study to derive the human cancer risk estimate	Lish et al. (1984) was a well-conducted study; five dose levels (including control) used, with a sufficient number of animals per dose group (at terminal sacrifice, n = 62–65 female mice/dose group for all groups besides the highest dose group). Tumor data from the mouse provided a stronger basis for estimating the OSF than rat data. Confidence in the OSF based on rat data was low because of the small numbers of tumors.
Species/sex Use of data sets from the male mouse or male rat would provide a lower OSF	OSF based on tumors in female B6C3F$_1$ mouse	It is assumed that a positive tumor response in animal cancer studies indicates that the agent can have carcinogenic potential in humans in the absence of data indicating that animal tumors are not relevant to humans (U.S. EPA, 2005a). As there are no data to inform whether the response in any given experimental animal species or sex would be most relevant for extrapolating to humans, tumor data from the most sensitive species and sex were selected as the basis for the OSF. Other data sets would provide smaller OSF values, and are not considered any more or less relevant to humans than data from the female mouse (i.e., 0.017 per mg/kg-day based on hepatocellular carcinomas in male F344 rats, and 0.027 per mg/kg-day based on alveolar/bronchiolar carcinomas in male B6C3F$_1$ mice; see Appendix D, Section D.2.3).
Combined tumor types Human risk would be underestimated if OSF was based on analysis using only a single tumor type	OSF based on liver and lung tumors in female B6C3F$_1$ mouse	Basing the OSF on one tumor site could potentially underestimate the carcinogenic potential of RDX, so an analysis that included data from the two tumor sites was chosen to calculate the combined risk (see Appendix D, Section D.2.1). Because there is no known biological dependence between the liver and lung tumors, independence between the two tumor sites was assumed. NRC (1994) considered the assumption of independence in incidence between tumor types to be reasonable when no evidence exists to the contrary.
Selection of dose metric PBPK models are available for the rat, mouse, and human, and using an appropriate internal metric can ↑ accuracy in human extrapolation	Mouse liver and lung tumors: administered dose used	EPA evaluated a published PBPK model in the mouse (Sweeney et al., 2012b); major uncertainties associated with limited toxicokinetic data in the mouse and unknown differences in metabolism across species were identified. Although EPA's preferred approach for extrapolating results from animal studies to humans is toxicokinetic modeling, the uncertainties associated with use of the mouse PBPK model for RDX were considered higher than use of administered dose.

Consideration and impact on cancer risk value	Decision	Justification
Cross-species scaling Alternatives could ↓ or ↑ OSF (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by $BW^{2/3}$])	$BW^{3/4}$ scaling (default approach)	There are no data to support alternatives. Because the dose metric was not an AUC, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. While the true human correspondence is unknown, this overall approach is not expected over- or underestimate human equivalent risks.
BMD model uncertainty Alternative models could ↓ or ↑ OSF	Use multistage model to derive a BMD and BMDL for combined tumor incidence	No biologically based models for RDX are available, and there is no a priori basis for selecting a model other than the multistage. The multistage model has biological support, as it allows for the statistical plausibility of low-dose linearity (see Appendix D, Section D.2.4), and is the model most consistently used in EPA cancer assessments (Gehlhaus et al., 2011). A sensitivity analysis using multistage and non-multistage models, with the highest dose dropped, revealed that the multistage models were the best-fitting or near best-fitting models for both liver and lung tumors.
Low-dose extrapolation approach ↓ cancer risk would be expected with the application of nonlinear extrapolation	Linear extrapolation from the POD	Where the available information is insufficient to establish the MOA for tumors at a given site, linear extrapolation is recommended because this extrapolation approach is generally considered to be health-protective (U.S. EPA, 2005a). Because the MOA for RDX-induced liver and lung tumors has not been established, linear low-dose extrapolation was applied, consistent with EPA guidance.
Statistical uncertainty at the POD ↓ OSF by 1.6-fold if BMD used as the POD rather than the BMDL	BMDL (default approach for calculating plausible upper bound OSF)	Lower bound is 95% CI on administered exposure at 10% ER of liver and lung tumors.
Sensitive subpopulations ↑ OSF to an unknown extent	Considered qualitatively	There is little information on whether some subpopulations may be more or less sensitive to the potential carcinogenicity of RDX (i.e., because of variability in toxicokinetics or toxicodynamics for RDX). The mode of carcinogenic action for liver and lung tumors in experimental animals is unknown, and little information is available on RDX metabolites or variation in metabolic rates that could be used to evaluate human variability in cancer response to RDX.
Historical control OSF changes no more than twofold if mean historical control tumor rates (from NTP) used rather than concurrent control rates	Concurrent control rate used in BMD modeling and to drive OSF	The concurrent control liver tumor rate (1.5%) was at the low end of the range (0–20%) for historical controls from NTP studies (Haseman et al., 1985). Concurrent control is generally preferred to historical control in BMD modeling, especially where historical control data come from a different laboratory. See Appendix D, Section D.2.4, and Table D-29.

2.3.5. Previous IRIS Assessment: Oral Slope Factor

The previous cancer assessment for RDX was posted to the IRIS database in 1990. The OSF in the previous cancer assessment was based on the bioassay by Lish et al. (1984) and analysis of data for hepatocellular adenomas or carcinomas in female mice. An OSF of 1.1×10^{-1} $(mg/kg\text{-}day)^{-1}$ was derived using a linearized multistage procedure (extra risk) and scaling by body weight to the 2/3 power for cross-species extrapolation. In addition, the previous assessment dropped the high-dose group because the dose was reduced at week 11 to address high mortality.

2.4. INHALATION UNIT RISK FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the inhalation unit risk (IUR) is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

An IUR value was not calculated because inhalation carcinogenicity data for RDX are not available. While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an IUR from the OSF.

2.5. APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS

As discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), either default or chemical-specific age-dependent adjustment factors (ADAFs) are recommended to account for early-life exposure to carcinogens that act through a mutagenic MOA. Because no chemical-specific data on lifestage susceptibility for RDX carcinogenicity are available, and because the MOA for RDX carcinogenicity is not known (see Section 1.2.7), application of ADAFs is not recommended.

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