EPA/635/R-16/208b
External Review Draft
www.epa.gov/iris

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

(CASRN 121-82-4)

Supplemental Information

September 2016

NOTICE

This document is an **External Review draft.** This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

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

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

CONTENTS

TABLES

FIGURES

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

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table A-1. Assessments by other national and international health agencies

Organization	Toxicity value
Agency for Toxic Substances and Disease Registry (ATSDR, 2012)	Acute oral Minimal Risk Level (MRL)—0.2 mg/kg-d Basis: tremors and convulsions in rats (Crouse et al., 2006); application of a composite uncertainty factor (UF) of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [physiologically based pharmacokinetic or PBPK modeling] and 10 for human variability) Intermediate oral MRL—0.1 mg/kg-d Basis: convulsions in rats (Crouse et al., 2006); application of a composite UF of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [PBPK modeling] and 10 for human variability) Chronic oral MRL—0.1 mg/kg-d Basis: tremors and convulsions in rats (Levine et al., 1983); application of a composite UF of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [PBPK modeling] and 10 for human variability)
National Institute for Occupational Safety and Health (NIOSH, 2012)	Recommended Exposure Limit (REL)—1.5 mg/m^3 TWA for up to a 10-hr workday during a 40-hr workweek; short-term (15-min) limit—3 mg/m^3 Basis: agreed with Occupational Safety and Health Administration (OSHA)-proposed Permissible Exposure Limit (PEL) in 1988 PEL Hearings Skin designation indicates potential for dermal absorption Basis: agreed with OSHA proposal for skin notation in 1988 PEL Hearings
Occupational Safety and Health Administration (OSHA, 2012a, b)	PEL—1.5 mg/m^3 time-weighted average (TWA) for an 8-hr workday in a 40-hr workweek Basis: adopted from the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) established in 1969 Skin designation indicates that cutaneous exposure may contribute to overall exposure and measures should be taken to prevent skin absorption Basis: adopted from ACGIH
Hazardous Substances Information System (Safe Work Australia, 2014)	Exposure standard—1.5 mg/m^3 TWA for an 8-hr workday Basis: adopted from the ACGIH TLV established in 1991 Skin absorption notice indicates that absorption through the skin may be a significant source of exposure Basis: adopted from ACGIH

APPENDIX B. ADDITIONAL DETAILS OF LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

The literature search for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was conducted in five online scientific databases through May 2016. The detailed search strategy used to search four of these databases—PubMed, Toxline, Toxcenter, and Toxic Substances Control Act Test Submissions (TSCATS)—is provided in Table B-1. Toxcenter, a fee-based scientific database, was searched outside of HERO. Toxcenter searches initially yield titles only; obtaining complete citations and abstracts incurs additional costs. Thus, titles only were initially screened; for titles identified as potentially relevant, complete citations with abstracts, when available, were downloaded and rescreened. Of the rescreened citations, only those selected for full text review were added to HERO and the RDX project page. The search strategy used to search the Defense Technical Information Center (DTIC) database is described in Table B-2. The computerized database searches were augmented by review of online regulatory sources, as well as "forward" and "backward" Web of Science searches of two recent reviews (Table B-3). Forward searching was used to identify articles that cited the selected studies (i.e., the two reviews identified in Table B-3), and backward searching was used to identify articles that the selected studies cited.

B.1. DEFENSE TECHNICAL INFORMATION CENTER (DTIC) LITERATURE SEARCH AND SCREEN

Among the RDX-related citations that were identified in the January 2015 search of the DTIC database, 826 (722 after duplicate removal within DTIC) were classified with the distribution "approved for public release", 239 (217 after duplicate removal) were classified as "distribution limited to U.S. Government agencies and their contractors," and 199 (181 after duplicate removal) were classified as "distribution limited to U.S. Government agencies only." A preliminary screen of the 1,120 unique citations was performed; 85 citations with unlimited distribution and 10 citations with limited distribution were selected for further review as potential sources of health effects data or supporting information. The remaining 1,025 unlimited and limited-distribution DTIC references not selected for further consideration were not studies of RDX or did not contain information pertinent to the assessment of the health effects of RDX (e.g., documents were related to environmental properties such as leaching, explosive properties, fuel and propellant properties, weapons systems, treatment of wastewater containing explosives, and disposal technologies). An update of the DTIC search was performed in May 2016. The update search identified 21 items

classified as "approved for public release," 9 classified as "distribution limited to U.S. Government agencies and their contractors," and 9 classified as "distribution limited to U.S. Government agencies only;" none of these was selected for further review, as none met the inclusion criteria outlined in Table LS-1 of the main document (i.e., none contained health effects data or supporting information).

The 85 unique selected citations with unlimited distribution were uploaded to the Health and Environmental Research Online (HERO) website[1] (http://hero.epa.gov). The 10 citations with limited distribution were subject to a more in-depth screen to determine whether the references provided additional primary health effects data and whether the U.S. Environmental Protection Agency (EPA) should seek authorization for public distribution and upload to HERO. A review of the abstract or full-text of the documents associated with the limited-distribution citations resulted in the following determinations:

- one citation was excluded because it did not provide additional primary health effects data. The citation reported data from a study that was subsequently published (Hathaway and Buck, 1977) and had already been identified by the literature search strategy.

- one citation (dated 1944) provided human and animal inhalation data and was considered pertinent, but was not brought forward for further review because flaws in the design of both the human and animal studies were such that results would not be considered credible. Experimental animal study design issues included lack of a control group, small numbers of animals, incomplete information on dosage or exposure levels, and inadequate reporting. The human study described a case series and lacked a referent group and measures of RDX exposure.

- eight citations were regulatory documents, reviews, or risk assessments that did not specifically identify RDX and did not appear to contain primary health effects data.

Based on these determinations, none of the 10 limited distribution citations that were subject to further review were selected for further consideration or added to HERO.

[1]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 1.6 million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO.

Table B-1. Summary of detailed search strategies for RDX (Pubmed, Toxline, Toxcenter, TSCATS)

Database	Terms	Hits
PubMed Date: 4/2012	((((121-82-4) OR (Cyclonite[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) OR (rdx[tw])) NOT medline[sb]) OR (((121-82-4) OR (Cyclonite[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) OR (rdx[tw])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND (humans[mh] OR animals[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR ((pharmacokinetics[mh] OR metabolism[mh]) AND (humans[mh] OR mammals[mh])) OR "dose-response relationship, drug"[mh] OR risk[mh] OR "toxicity tests"[mh] OR noxae[mh] OR cancer[sb] OR "endocrine system"[mh] OR "endocrine disruptors"[mh] OR "Hormones, Hormone Substitutes, and Hormone Antagonists"[mh] OR triazines/ai OR ("Inhalation Exposure"[Mesh] OR "Maternal Exposure"[Mesh] OR "Maximum Allowable Concentration"[Mesh] OR "Occupational Exposure"[Mesh] OR "Paternal Exposure"[Mesh] OR "Environmental Exposure"[Mesh:noexp]))))) NOT (((((121-82-4) OR (Cyclonite[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-	337

Database	Terms	Hits
	1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) OR (rdx[tw])) NOT medline[sb]) OR (((121-82-4) OR (Cyclonite[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) OR (rdx[tw])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND (humans[mh] OR animals[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR ((pharmacokinetics[mh] OR metabolism[mh]) AND (humans[mh] OR mammals[mh])) OR "dose-response relationship, drug"[mh] OR risk[mh] OR "toxicity tests"[mh] OR noxae[mh] OR cancer[sb] OR "endocrine system"[mh] OR "endocrine disruptors"[mh] OR "Hormones, Hormone Substitutes, and Hormone Antagonists"[mh] OR triazines/ai OR ("Inhalation Exposure"[Mesh] OR "Maternal Exposure"[Mesh] OR "Maximum Allowable Concentration"[Mesh] OR "Occupational Exposure"[Mesh] OR "Paternal Exposure"[Mesh] OR "Environmental Exposure"[Mesh:noexp])))) AND (invertebrates OR aquatic organisms OR fish OR fishes OR amphibians OR earthworm*))	
PubMed Date limit: 1/2012–2/2013	(Cyclonite[tw] OR RDX[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) AND (("2012/01/01"[Date - MeSH] : "3000"[Date - MeSH]) OR ("2012/01/01"[Date - Entrez] : "3000"[Date - Entrez]) OR ("2012/01/01"[Date - Create] : "3000"[Date - Create]))	112

Database	Terms	Hits
PubMed Date limit: 11/2012–1/2014	(Cyclonite[tw] OR RDX[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) AND (("2012/11/01"[Date - MeSH] : "3000"[Date - MeSH]) OR ("2012/11/01"[Date - Entrez] : "3000"[Date - Entrez]) OR ("2012/11/01"[Date - Create] : "3000"[Date - Create]))	138
PubMed Date limit: 11/2013–1/2015	("cyclonite"[nm] OR Cyclonite[tw] OR RDX[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) AND (2013/11/01 : 3000[crdat] OR 2013/11/01 : 3000[edat])	76
PubMed Date limit: 11/2014–5/2016	("cyclonite"[nm] OR Cyclonite[tw] OR RDX[tw] OR Cyclotrimethylenetrinitramine[tw] OR "cyclotrimethylene trinitramine"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR "Hexahydro-1,3,5-trinitro-s-triazine"[tw] OR Hexogen[tw] OR "1,3,5-trinitro-1,3,5-triazine"[tw] OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane"[tw] OR "1,3,5-Trinitro-1,3,5-triazacyclohexane"[tw] OR "1,3,5-Trinitrohexahydro-1,3,5-triazine"[tw] OR "1,3,5-Trinitrohexahydro-s-triazine"[tw] OR "1,3,5-Trinitroperhydro-1,3,5-triazine"[tw] OR "Esaidro-1,3,5-trinitro-1,3,5-triazina"[tw] OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin"[tw] OR "Perhydro-1,3,5-trinitro-1,3,5-triazine"[tw] OR Cyclotrimethylenenitramine[tw] OR Trimethylenetrinitramine[tw] OR "Trimethylene trinitramine"[tw] OR Trimethyleentrinitramine[tw] OR "Trinitrocyclotrimethylene triamine"[tw] OR Trinitrotrimethylenetriamine[tw] OR "CX 84A"[tw] OR Cyklonit[tw] OR Geksogen[tw] OR Heksogen[tw] OR Hexogeen[tw] OR Hexolite[tw] OR "KHP 281"[tw] OR "PBX (af) 108"[tw] OR "PBXW 108(E)"[tw] OR "Pbx(AF) 108"[tw]) AND (2014/11/01 : 3000[crdat] OR 2014/11/01 : 3000[edat])	118

Database	Terms	Hits
Toxline Date: 4/2012	Notes: Searched CASRN or synonyms; removed invertebrates, aquatic organisms, amphibians, earthworms.	507
Toxline Date limit: 2011–2/2013	@OR+("Cyclonite"+"RDX"+"Cyclotrimethylenetrinitramine"+"cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"1,3,5-trinitro-1,3,5triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+@term+@rn+121-82-4)+@AND+@range+yr+2011+2013+@NOT+@org+pubmed+pubdart+crisp+tscats	5
	@OR+("1,3,5-Trinitroperhydro-1,3,5-triazine"+"Esaidro-1,3,5-trinitro-1,3,5-triazina"+"Hexahydro-1,3,5-trinitro-1,3,5-triazin"+"Perhydro-1,3,5-trinitro-1,3,5-triazine"+"Cyclotrimethylenenitramine"+"Trimethylenetrinitramine"+"Trimethylene+trinitramine"+"Trimethyleentrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"CX+84A"+"Cyklonit"+"Geksogen"+"Heksogen"+"Hexogeen"+"Hexolite"+"KHP+281")+@AND+@range+yr+2011+2013+@NOT+@org+pubmed+pubdart+crisp+tscats	0
Toxline Date limit: 2012–1/2014	@OR+("Cyclonite"+"RDX"+"Cyclotrimethylenetrinitramine"+"cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"1,3,5-trinitro-1,3,5-triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+@term+@rn+121-82-4)+ @AND+@range+yr+2012+2014+@NOT+@org+pubmed+pubdart+crisp+tscats	10
	@OR+("1,3,5-Trinitroperhydro-1,3,5-triazine"+"Esaidro-1,3,5-trinitro-1,3,5-triazina"+"Hexahydro-1,3,5-trinitro-1,3,5-triazin"+"Perhydro-1,3,5-trinitro-1,3,5-triazine"+"Cyclotrimethylenenitramine"+"Trimethylenetrinitramine"+"Trimethylene+trinitramine"+"Trimethyleentrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"CX+84A"+"Cyklonit"+"Geksogen"+"Heksogen"+"Hexogeen"+"Hexolite"+"KHP+281")+@AND+@range+yr+2012+2014+@NOT+@org+pubmed+pubdart+crisp+tscats	0
Toxline Date limit: 2013-1/2015	@SYN0+@OR+(RDX+"cyclotrimethylene+trinitramine"+"1,3,5-trinitro-1,3,5-triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+"1,3,5-Trinitroperhydro-1,3,5-triazine"+"CX+84A")+@AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	19
	@SYN0+@OR+("Cyclonite"+"Cyclotrimethylenenitramine"+"Cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"Hexolite"+"KHP+281"+"PBX+(af)+108"+"PBXW+108(E)"+"Pbx(AF)+108"+"Perhydro-1,3,5-trinitro-1,3,5-triazine")+@AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	9
	@SYN0+@OR+("Research+Development+Explosive"+"Royal+Demolition+eXplosive+"Trimethylenetrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"sym-Trimethylene+trinitramine"+@term+	0

Database	Terms	Hits
	@rn+121-82-4+@term+@rn+204655-61-8+@term+@rn+50579-23-2+@term+@rn+53800-53-6+@term+@rn+57608-45-4+@term+@rn+82030-42-0)+ @AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	
Toxline Date limit: 2014-5/2016	@SYN0+@OR+(RDX+"cyclotrimethylene+trinitramine"+"1,3,5-trinitro-1,3,5-triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+"1,3,5-Trinitroperhydro-1,3,5-triazine"+"CX+84A")+@AND+@range+yr+2014+2016+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	1
	@SYN0+@OR+("Cyclonite"+"Cyclotrimethylenenitramine"+"Cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"Hexolite"+"KHP+281"+"PBX+(af)+108"+"PBXW+108(E)"+"Pbx(AF)+108"+"Perhydro-1,3,5-trinitro-1,3,5-triazine")+@AND+@range+yr+2014+2016+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	0
	@SYN0+@OR+("Research+Development+Explosive"+"Royal+Demolition+eXplosive+"Trimethylenetrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"sym-Trimethylene+trinitramine"+@term+@rn+121-82-4+@term+@rn+204655-61-8+@term+@rn+50579-23-2+@term+@rn+53800-53-6+@term+@rn+57608-45-4+@term+@rn+82030-42-0)+@AND+@range+yr+2014+2016+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp	0
TSCATS Date: 2/2013	@term+@rn+121-82-4+@AND+@org+tscats	4
TSCATS 2 Date: 5/2016	121-82-4 from EPA receipt date 01/01/2000	0
TSCATS 8e/FYI **Date:** 5/2016	("121-82-4" OR "1,3,5-Triazine, hexahydro-1,3,5-trinitro-") tsca (8e OR FYI)	0

Database	Terms	Hits
Toxcenter Date: 4/2012	((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs))AND ((chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct,it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND ((biosis/fs AND py>1999) OR caplus/fs)) Notes: Duplicates were removed; Biosis subfile results were date limited to avoid extensive overlap with Toxline.	337 titles screened (20 selected for full records and added to HERO)
Toxcenter Date limit: 1/2012–2/2013	(((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene	26 titles screened (6 selected for full records and added to HERO)

Database	Terms	Hits
	trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py>2012 OR ed>20120101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (biosis/fs OR caplus/fs)) Notes: Duplicates were removed.	
Toxcenter Date limit: 11/2012–1/2014	(((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py>2012 OR ed>20121101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR	20 titles screened (0 selected for full records; none added to HERO)

Database	Terms	Hits
	(maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (biosis/fs OR caplus/fs)) Notes: Duplicates were removed.	
Toxcenter Date limit: 11/2013–1/2015	(((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py >2013 OR ed>20131101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous?	80 titles screened (3 selected for full records and added to HERO)

Database	Terms	Hits
	OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (biosis/fs OR caplus/fs)) Note: Duplicates were removed.	
Toxcenter Date limit: 11/2014–5/2016	(((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5 triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5 triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py >2013 OR ed>20131101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous? OR carcinog? OR cocarcinog? OR cancer? OR precancer? OR neoplas? OR tumor? OR tumour? OR oncogen? OR lymphoma? OR carcinom? OR genetox? OR genotox? OR mutagen? OR genetic(w)toxic? OR nephrotox? OR hepatotox? OR endocrin? OR estrogen? OR androgen? OR hormon? OR rat OR rats OR mouse OR mice OR muridae OR dog OR dogs OR rabbit? OR hamster? OR pig OR pigs OR swine OR porcine OR goat OR goats OR sheep OR monkey? OR macaque? OR marmoset? OR primate? OR mammal? OR ferret? OR gerbil? OR rodent? OR lagomorpha OR baboon? OR bovine OR canine OR cat OR cats OR feline OR pigeon? OR occupation? OR worker? OR epidem?) AND (biosis/fs OR caplus/fs))	33 titles screened (0 selected for full records and added to HERO)

Database	Terms	Hits
	Note: Duplicates were removed.	

Table B-2. Summary of detailed search strategies for RDX (DTIC)

Database	Terms	Hits
DTIC Online Access Controlled Date searched: 1/2015	Synonyms in all fields search box ("121-82-4" OR "RDX" OR "Cyclotrimethylenetrinitramine" OR "Cyclonite" OR "cyclotrimethylene trinitramine" OR "Hexogen" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR "Trimethylene trinitramine" OR "Trimethylenetrinitramine" OR "Hexolite" OR "Trinitrotrimethylenetriamine") Keywords in citation box ("toxicity" OR "toxicology" OR "poisoning" OR "cancer" OR "carcinogens" OR "carcinogen" OR "neoplasms" OR "neoplasm" OR "oncogenesis" OR "teratogenic compounds" OR "lethality" OR "death" OR "body weight" OR "immunology" OR "genotoxicity" OR "mutagenicity" OR "mutagens" OR "mutations" OR "oral" OR "gavage" OR "inhalation" OR "dermal" OR "metabolism" OR "pharmacokinetics" OR "pharmacokinetic" OR "PBPK" OR "pharmacology" OR "organs" OR "skin" OR "tissues" OR "body fluids" OR "toxic agents" OR "rats" OR "mice" OR "mouse" OR "rat") Limited to Content type: Documents	
	Distribution: Approved for Public Release	826 (85 selected and added to HERO)
	Distribution: U.S. Gov't and Contractors	239 (0 selected and added to HERO)
	Distribution: U.S. Gov't Only	199 (0 selected and added to HERO)
DTIC Online Access Controlled Date searched: 5/2016	Synonyms in all fields search box ("121-82-4" OR "RDX" OR "Cyclotrimethylenetrinitramine" OR "Cyclonite" OR "cyclotrimethylene trinitramine" OR "Hexogen" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR "Trimethylene trinitramine" OR "Trimethylenetrinitramine" OR "Hexolite" OR "Trinitrotrimethylenetriamine") Keywords in citation box ("toxicity" OR "toxicology" OR "poisoning" OR "cancer" OR "carcinogens" OR "carcinogen" OR "neoplasms" OR "neoplasm" OR "oncogenesis" OR "teratogenic compounds" OR "lethality" OR "death" OR "body weight" OR "immunology" OR "genotoxicity" OR "mutagenicity" OR "mutagens" OR "mutations" OR "oral" OR "gavage" OR "inhalation" OR "dermal" OR "metabolism" OR "pharmacokinetics" OR "pharmacokinetic" OR "PBPK" OR "pharmacology" OR "organs" OR "skin" OR "tissues" OR "body fluids" OR "toxic agents" OR "rats" OR "mice" OR "mouse" OR "rat") Limited to Content type: Documents	

Database	Terms	Hits
	Distribution: Approved for Public Release	21 (0 selected and added to HERO)
	Distribution: U.S. Gov't and Contractors	9 (0 selected and added to HERO)
	Distribution: U.S. Gov't Only	9 (0 selected and added to HERO)

Table B-3. Processes used to augment the search of core databases for RDX

Selected key reference(s) or sources	Date	Additional references identified
"Forward" and "backward" Web of Science searches[a]		
Sweeney et al. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling. Regul Toxicol Pharmacol 62(1):107−114. (*forward search*) 1 search result	3/2013	0 citations added
Sweeney et al. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64(2):205−224 (*backwards search*) 0 search results	3/2013	0 citations added
Sweeney et al. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling. Regul Toxicol Pharmacol 62(1):107−114. (*review of 35 references cited in this paper*)	3/2013	0 citations added
Sweeney et al. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64(2):205−224 (*review of 69 references cited in this paper*)	3/2013	3 citations added
Online regulatory sources		
Combination of Chemical Abstracts Registry Number (CASRN) and synonyms searched on the following websites: Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov/substances/index.asp (Note: the reference list for the ATSDR toxicological profile for RDX was compared to the search results and relevant references were added) California Environmental Protection Agency (Office of Environmental Health Hazard Assessment) (http://www.oehha.ca.gov/risk.html) eChemPortal (http://www.echemportal.org/echemportal/participant/page.action?pageID=9) EPA Acute Exposure Guideline Levels (http://www.epa.gov/oppt/aegl/pubs/chemlist.htm) (http://www.epa.gov/ncea/iris/index.html) to find data EPA National Service Center for Environmental Publications (NSCEP) (http://www.epa.gov/ncepihom/) EPA Science Inventory	4/2012 1/2014 1/2015 5/2016	15 citations added 1 citation added 0 citations added 0 citations added

Selected key reference(s) or sources	Date	Additional references identified
(http://cfpub.epa.gov/si/) Federal Docket www.regulations.gov Health Canada First Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php) Health Canada Second Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php) International Agency for Research on Cancer (IARC) (http://monographs.iarc.fr/htdig/search.html) International Programme on Chemical Safety (IPCS) INCHEM (http://www.inchem.org/) National Academy of Science (NAS) via the National Academies Press (http://www.nap.edu/) National Cancer Institute (NCI) (http://www.cancer.gov) National Center for Toxicological Research (NCTR) (http://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/default.htm) National Institute of Environmental Health Sciences (NIEHS) (http://www.niehs.nih.gov/) National Institute for Occupational Safety and Health (NIOSH) NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) National Toxicology Program (NTP)—RoC, status, results, and management reports (http://ntpsearch.niehs.nih.gov/query.html) World Health Organization (WHO) assessments—Concise International Chemical Assessment Documents (CICADs), Environmental Health Criteria (EHC) (http://www.who.int/ipcs/assessment/en/)		

[a]Sweeney et al. (2012a) and Sweeney et al. (2012b) were selected for forward and backward searching in the Web of Science as the two more recent reviews of the health effects of RDX toxicity in the published literature.

APPENDIX C. INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ANALYSIS

C.1. TOXICOKINETICS

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is absorbed following exposure by inhalation and oral routes. The rate and extent of absorption are dependent upon the dosing preparation. RDX is systemically distributed, can be transferred from mother to fetus, and can transfer in maternal milk. Metabolism of RDX is extensive and includes denitration, ring cleavage, and generation of CO_2 possibly through cytochrome P450 (CYP450). RDX metabolites are eliminated primarily via urinary excretion and exhalation of CO_2.

C.1.1. Absorption

Absorption of RDX following oral exposure has been demonstrated in humans and laboratory animals (rats, mice, swine, and voles) through measurement of radiolabeled RDX and/or metabolites in excreta (urine and expired air) and tissues (including blood). Quantitative estimates of oral absorption (e.g., oral bioavailability or fractional absorption) are not available in humans. Results of animals studies indicate that oral bioavailability ranges from approximately 50 to 90% and may vary based on the physical form of RDX and the matrix (e.g., soil, plants) in which it is administered. Studies investigating absorption of RDX following inhalation exposure were not identified. Results of an intratracheal administration study in rats provide limited evidence of absorption of RDX from the respiratory tract. The only data evaluating dermal absorption of RDX is provided by in vitro studies showing that RDX can be absorbed through excised skin of humans and animals.

Oral Absorption

Quantitative information on blood levels following accidental exposure to RDX is limited to two studies of accidental oral exposures (Küçükardali et al., 2003; Woody et al., 1986) and one study of mixed dermal and inhalation exposure (Özhan et al., 2003). A number of qualitative case studies of accidental exposures with similar toxic effects provide additional support that RDX is absorbed into the body (Hett and Fichtner, 2002; Harrell-Bruder and Hutchins, 1995; Goldberg et al., 1992; Ketel and Hughes, 1972; Hollander and Colbach, 1969; Stone et al., 1969). The oral absorption of RDX in humans was demonstrated in a case report of a 3-year-old male child who ingested plasticized RDX material that adhered to his mother's work boots and clothing (Woody et

al., 1986). RDX was measured in serum, urine, cerebrospinal fluid, and feces. Based on a kinetic analysis of the serum RDX concentrations, the dose was estimated to be 85 mg/kg and the first-order absorption rate constants were estimated to be 0.34–2.20 hour^{-1} (Woody et al., 1986)[2]. Sweeney et al. (2012a) estimated the absorption rate constant for this same subject to be 0.060 hour^{-1}. The large range in the calculated absorption rate constants resulted from uncertainty in the dose and time to peak serum RDX levels, and the models that were used to simulate the RDX toxicokinetics. Özhan et al. (2003) summarized plasma RDX levels in five military personnel who were accidentally exposed to toxic levels of RDX. Although Özhan et al. (2003) reported that personnel were exposed through dermal and inhaled routes, secondary oral exposure may have occurred. Based on physiologically based pharmacokinetic (PBPK) model fits to the plasma RDX concentration data, Sweeney et al. (2012a) estimated a first-order absorption rate constant of 0.033 hour^{-1}. Küçükardali et al. (2003) summarized plasma RDX levels in five military personnel who ingested toxic levels of RDX (doses were not reported). RDX was detected in plasma of all patients within 3 hours after ingestion.

Quantitative data to directly support estimates of oral bioavailability are available from studies in rats and mice (Guo et al., 1985; Schneider et al., 1978, 1977). Results of single and repeated oral dose studies in adult Sprague-Dawley rats indicate that approximately 83–87% of the administered dose is absorbed from the gastrointestinal (GI) tract. Following gavage administration of 50 mg/kg [^{14}C]-RDX dissolved in dimethylsulfoxide (DMSO), approximately 90% of the administered carbon-14 was recovered 4 days after dosing, with ~3% in feces, 34% in urine, 43% in expired air, and 10% in the carcass (Schneider et al., 1977). It is unclear if the carcass includes the GI tract, which may include unabsorbed RDX. Assuming that all of the carbon-14 in feces represents unabsorbed RDX (rather than RDX that was absorbed and subsequently secreted into the intestine), results of this study indicate that at least 87% of the administered dose was absorbed from the GI tract. Similar results were observed following repeated daily oral exposure of Sprague-Dawley rats to [^{14}C]-RDX by gavage (in DMSO) or drinking water for 1 week. Based on recovery of carbon-14 in urine and expired air and the carbon-14 retained in carcass, approximately 83% (drinking water) to 85 % (gavage) of the administered dose was absorbed (Schneider et al., 1978).

An estimate of oral bioavailability in rats can also be obtained from data on blood RDX concentrations reported in Krishnan et al. (2009). Male Sprague-Dawley rats received a single intravenous (i.v.) (0.77 or 1.04 mg/kg) or oral (1.53 or 2.07 mg/kg, dissolved in water) dose of RDX. Estimates of bioavailability were obtained based on the reported blood RDX concentrations, terminal elimination rate constants (estimated for this review by fitting the serum RDX data with a first-order exponential function, see Table C-5 in Section C.1.4, Excretion) and the blood area under the curve (AUC) values (calculated for this review using the trapezoid rule extrapolated to infinite

[2]Woody et al. (1986) reported the absorption rate constants in units of L/hour; however, this appears to have been a typographical error for 1/hour or hour^{-1}.

time). Calculated dose-adjusted AUC values were 9.6 and 8.4 hours-kg/L for the i.v. studies and 4.7 and 6.0 hours-kg/L for the oral dosing studies. These AUC values correspond to estimated oral bioavailability ranging from 50 to 70%. Recovery of administered radiolabel was incomplete (~90% of the administered carbon-14) in the studies (Schneider et al., 1978, 1977); therefore, it is possible that oral bioavailability is actually higher than 83–87%. Guo et al. (1985) reported data on blood tritium kinetics in mice that received i.v. (0.055 mg RDX or ~2.5 mg/kg body weight) or oral (50 mg/kg) doses of [^{3}H]-RDX. Based on the reported blood tritium concentrations (% of dose/g) and terminal $t_{1/2}$ values for concentrations of tritium in blood (1.1 days for i.v. and 2.2 days for oral), the corresponding AUCs of the blood concentration versus time curves were calculated (calculated for this review using the trapezoid rule extrapolated to infinite time) to be 30 and 16 hours-% dose/g for i.v. and oral dosing, respectively. This corresponds to an oral bioavailability of RDX-derived tritium concentration of approximately 50% (i.e., 16/30).

In Yucatan miniature swine administered a single dose of [^{14}C]-RDX (43–45 mg/kg as a suspension in carboxymethylcellulose), approximately 0.8–6% of the administered carbon-14 was eliminated in feces 24 hours after dosing (Musick et al., 2010; Major et al., 2007). Although results of swine studies suggest that GI absorption of RDX was nearly complete, data cannot be used to determine a quantitative estimate of oral bioavailability because it is unlikely that fecal excretion of unabsorbed RDX was complete 24 hours after dosing (Snoeck et al., 2004).

Oral bioavailability of RDX appears to vary depending upon the physical form of RDX and the matrix (e.g., soil, vegetation) in which it is administered. Schneider et al. (1977) compared the oral absorption of a single 100 mg/kg gavage dose of coarse granular [^{14}C]-RDX as a slurry in isotonic saline with a single 50 mg/kg gavage dose of a finely powdered [^{14}C]-RDX solution in saline in Sprague-Dawley rats. Plasma carbon-14 levels were measured for 24 hours after dosing. For both [^{14}C]-RDX preparations, peak plasma levels of carbon-14 were observed 24 hours after oral administration, with a higher 24-hour plasma concentration for the 50 mg/kg dose (~4.7 µg/mL) compared to the 100 mg/kg dose (3.12 µg/mL). Results of this study indicate that the oral bioavailability of RDX may be greater for the finely powdered preparation than for the coarse granular preparation consistent with a greater surface area available for absorption with finely powdered RDX. However, blood levels were only measured 24 hours after dosing, and the lower 24-hour carbon-14 plasma concentration for the coarse granular preparation could be due to slower absorption of coarse RDX granules compared with fine RDX powder, rather than lower overall bioavailability.

Oral bioavailability of RDX is lower when administered as RDX-contaminated soil or when RDX is in plant materials that were grown on RDX-contaminated soils. Crouse et al. (2008) investigated the oral bioavailability of RDX in contaminated soils relative to pure RDX by comparison of the AUC for the RDX blood concentration versus time curves. Adult male Sprague-Dawley rats were administered oral doses (in gelatin capsules) of pure RDX (99.9% purity; neat) or an equivalent amount of RDX in contaminated soils from the Louisiana Army Ammunition

Plant (AAP) or Fort Meade. Blood concentrations for rats dosed with Louisiana AAP soil (1.24 mg/kg) and neat RDX (1.24 mg/kg) peaked at approximately 6 hours. The AUC and 6-hour RDX blood concentration were both approximately 25% lower for Louisiana AAP soil than for neat RDX ($p \leq 0.003$ for AUC), suggesting that oral bioavailability of RDX from Louisiana AAP soil was 25% lower than neat RDX. For Fort Meade soil (0.2 mg/kg), RDX blood concentrations peaked at 6 hours compared to 4 hours for neat RDX (0.2 mg/kg). The 4-hour blood concentration for Fort Meade soil was approximately 15% lower than for neat RDX, although the AUC for Fort Meade soil was only 5% lower than for neat RDX (not statistically significant). Collectively, these results suggest that RDX in soil is absorbed following oral exposure and that it has a lower bioavailability than neat RDX.

Fellows et al. (2006) showed that plants (alfalfa shoots and corn leaves) incorporated [^{14}C]-RDX grown on [^{14}C]-RDX-amended soils. [^{14}C]-RDX and plant metabolites of [^{14}C]-RDX were absorbed by voles following oral administration (Fellows et al., 2006). In adult male prairie voles (*Microtus ochrogaster*) fed diets containing RDX incorporated in plants for 5 or 7 days (average RDX dose per animal of 2.3 mg/kg-day), 94.8 and 96.6% (respectively) of the administered carbon-14 was eliminated in excreta (combined feces, urine, and CO_2) and 3–5% was retained in the carcass. Feces, urine, and CO_2 contained 74–79, 13–14, and 8–12% of the total carbon-14 in excreta, respectively. Based on carbon-14 elimination in urine and CO_2 plus that retained by the carcass, the study authors estimated the oral bioavailability of plant-derived RDX to be >20%. However, if biliary excretion of RDX and/or RDX metabolites is a major excretory pathway in voles (as is the case with mice), estimates of bioavailability of plant-derived RDX could be substantially higher.

In Yorkshire piglets administered single doses of 5 or 10 mg/kg in gelatin capsules, peak plasma concentrations were proportional to the administered dose (Bannon, 2006). However, the potential for dose-dependence has not been evaluated over a wide range of doses.

RDX appears in blood within 1 hour following oral dosing; however, the rate of absorption may depend upon the physical form or dose of RDX (Bannon et al., 2009a; Crouse et al., 2008; Bannon, 2006; Guo et al., 1985; MacPhail et al., 1985; Schneider et al., 1977). Oral absorption of RDX was rapid in LACA mice following stomach perfusion with [^{3}H]-RDX (50 mg/kg in methyl cellulose) (Guo et al., 1985). The tritium radiolabel was detected in blood 15 minutes following dosing and the highest concentrations in blood were observed 30 minutes after dosing. Based on an analysis of the blood tritium concentration kinetics, the authors estimated an absorption rate constant of 8.7 hour^{-1}. In Sprague-Dawley rats administered single doses (0.2–18.0 mg/kg) of RDX in gelatin capsules, peak blood RDX concentrations were observed between 2.5 and 6 hours (Bannon et al., 2009a; Krishnan et al., 2009; Crouse et al., 2008). Peak blood concentrations occurred at 24 hours after Sprague-Dawley rats were administered a single oral dose (100 mg/kg) of coarse granular RDX in saline (Schneider et al., 1977). Similarly, peak RDX blood concentrations in swine administered single doses (5–10 mg/kg) of finally powdered (>98% pure) RDX in gelatin capsules occurred at 3–8 hours after dosing (Bannon et al., 2009a), compared to 24 hours after a

single dose (100 mg/kg) of RDX administered as a finely powdered in saline (Bannon et al., 2009a; Schneider et al., 1977). Peak plasma concentrations in Yucatan miniature swine administered a single dose of [^{14}C]-RDX (45 mg/kg as a suspension in carboxymethylcellulose) were reached within 6−12 hours after dosing (Musick et al., 2010). Krishnan et al. (2009) and Sweeney et al. (2012a) estimated absorption rates in rats dosed with higher doses of coarse granular RDX to be approximately 100 times slower than absorption rates in rats dosed with lower doses of finely powdered neat RDX preparations or neat RDX dissolved in aqueous vehicles. For example, Krishnan et al. (2009) estimated the absorption rate constant to be 0.75 hour^{-1} for rats dosed with neat RDX dissolved in water (1.53 or 2.07 mg/kg) or neat RDX in a gelatin capsule (0.2 or 1.24 mg/kg) (Crouse et al., 2008), compared to 0.0075 hour^{-1} for rats dosed with coarse granular RDX (100 mg/kg) (Schneider et al., 1977).

Inhalation Absorption

Studies evaluating absorption of RDX in humans following inhalation exposure were not identified. Several case reports have documented seizures and other neurological effects in individuals exposed to RDX either in a manufacturing setting or in the course of using RDX as a cooking fuel (Testud et al., 1996a; Testud et al., 1996b; Ketel and Hughes, 1972; Hollander and Colbach, 1969; Kaplan et al., 1965; Barsotti and Crotti, 1949). These reports suggest that RDX may be absorbed by the respiratory system. However, in several cases, the study authors were unable to clearly identify the primary route of exposure. In some cases, incidental oral exposure was suggested. Studies in laboratory animals have not investigated the absorption of RDX following inhalation exposure.

Dermal Absorption

In vitro studies have demonstrated the dermal absorption of RDX in human and pig skin (Reddy et al., 2008; Reifenrath et al., 2008). Reddy et al. (2008) reported that 5.7% of the applied RDX dose (in acetone) was absorbed into excised human skin in 24 hours. Dermal absorption of [^{14}C]-RDX from both a low-carbon (1.9%) and a high-carbon (9.5%) soil was also assessed in this system. Approximately 2.6% of the RDX applied in the low-carbon soil and 1.4% applied in the high-carbon soil was absorbed after 24 hours. Thus, the dermal absorption of RDX from soils was reduced when compared with absorption from acetone, and absorption was lower in the high-carbon soil than in the low-carbon soil.

Reifenrath et al. (2008) investigated the influence of skin surface moisture conditions, soil carbon content, and soil aging on the in vitro percutaneous penetration of [^{14}C]-labeled RDX in excised pig skin. Mean skin absorption of RDX was higher for low-carbon soil (1.2%), regardless of soil age and skin surface moisture. Absorption and evaporation were <1% for RDX regardless of soil type and age. While dermal absorption of certain munitions (e.g., 2,6-dinitrotoluene) is greatly enhanced by hydration of the skin surface, hydration had minimal effect on RDX, mostly due to the lack of RDX volatility (e.g., <0.5% evaporation).

C.1.2. Distribution

Information on the distribution of absorbed RDX in humans is limited to a few cases of accidental exposures to RDX that provide data on the kinetics of RDX in blood and cerebrospinal fluid (Küçükardali et al., 2003; Özhan et al., 2003; Woody et al., 1986). Concentrations of RDX in serum and cerebrospinal fluid were similar (11 and 9 mg/L, respectively) in a child 24 hours after ingesting an estimated dose of 85 mg/kg RDX (Woody et al., 1986). More extensive information on tissue distribution is available for animals, including mice, rats, and swine (Musick et al., 2010; Bannon, 2006; Reddy et al., 1989; Guo et al., 1985; MacPhail et al., 1985; Schneider et al., 1977). In these studies, RDX or radiolabeled RDX ([^{14}C] or [^{3}H) was administered by the oral, intraperitoneal (i.p.), i.v., or intratracheal route and the distribution of the RDX or radiolabel was measured. Since metabolism of RDX can result in loss of carbon-14 or tritium from the parent compound, the distribution of radiolabel will not necessarily reflect the distribution of RDX (Schneider et al., 1977). To compare tissue distributions in studies in which animals received different doses by different routes of administration, distribution data are expressed as ratios of tissue RDX or radiolabel to that of either whole blood or plasma, whichever was reported. RDX in blood distributes into red blood cells (RBCs) and plasma to achieve concentration ratios that are close to unity. The plasma:whole blood carbon-14 ratio in swine that received a single oral dose of [^{14}C]-RDX (45 mg/kg) was approximately 1.3 (Musick et al., 2010), and whole rat blood incubated in vitro with RDX had a plasma:RBC RDX ratio of approximately 1.0 (Krishnan et al., 2009). As a result of the similarity between plasma and whole blood concentrations, tissue distribution is approximately equivalent when expressed as ratios of blood or plasma.

Studies conducted in rats, mice, and swine indicate that absorbed RDX distributes to many different tissues. Schneider et al. (1977) estimated the volume of distribution of RDX to be approximately 2.18 L/kg in rats, based on plasma RDX kinetics in rats that received a single i.p. dose of RDX (5–6 mg/kg). Consistent with this estimate are observations of tissue:blood (or plasma) concentration ratios that exceed 1 in various tissues, including brain (showing that RDX can cross the blood:brain barrier), heart, kidney, and liver (Musick et al., 2010; Bannon et al., 2006; MacPhail et al., 1985; Schneider et al., 1977). Distribution within the brain may not be uniform. However, Bannon et al. (2006) observed tissue:blood concentrations for RDX of approximately 4 in brain hippocampus and 3.5 in brain cortex of swine that received a single oral dose of 10 mg/kg [^{14}C]-RDX, although this is the only study that reported distribution for brain regions. Reported tissue:blood (or plasma) concentration ratios of RDX 24 hours following a single dose (oral or i.p.) were 1–9 for kidney, 1–7 for liver, and 1–3 for heart (Table C-1) (Bannon, 2006; Schneider et al., 1977). With repeated oral dosing (e.g., 30–90 days), tissue:blood ratios of RDX for these tissues are consistently greater than unity (Schneider et al., 1978). There is no consistent evidence that RDX accumulates in fat, although estimates of the fat:blood partition coefficient range from 6 to 8 and exceed that of other tissues (Sweeney et al., 2012a; Krishnan et al., 2009).

Table C-1. Distribution of RDX or radiolabel from administered RDX[a]

Animal	Route	Dose (mg/kg)	Time (hrs)	Brain	Heart	Kidney	Liver	Fat	Source
Swine	Oral	45[b]	24	0.6[c]	0.7	2.4	7.3	0.4	Musick et al. (2010)
Swine	Oral	10[d]	3	3.5–4.0[d]	2	≤1	<1	NA[g]	Bannon et al. (2006)
Swine	Oral	100[d]	24	1.5[c]	1.1	1.2–1.9	0.9	1.8	Schneider et al. (1977)
Rat	Oral	100[d]	24	3.4[c]	2.9	6.6	0.7	NA	Schneider et al. (1977)
Rat	i.p.	50[d]	2	3.4[c]	2.6	8.8	5.7	NA	Schneider et al. (1977)
Rat	i.p.	500[d]	≤6.5	2.5[c]	2.1	4.8	3.3	NA	Schneider et al. (1977)
Mouse	Oral	50[e]	24	1[c]	0.8	1	1.4	0.8	Guo et al. (1985)
Mouse	i.v.	2.5[e]	24	0.6[f]	0.8	0.7	1.6	0.4	Guo et al. (1985)

[a]Values are tissue:blood or tissue:plasma ratios following a single dose of either RDX, [^{14}C]-RDX, or [^{3}H]-RDX.
[b]Carbon-14
[c]Tissue:plasma
[d]RDX
[e]Tritium
[f]Tissue:blood
[g]Not available

In rats, RDX can cross the placental:blood barrier resulting in exposure to the fetus, and can also be transported into maternal milk. Hess-Ruth et al. (2007) detected RDX in the brain tissue of postnatal day (PND) 1 rat pups (concentrations ranged from 0.64 to 7.6 µg/g brain tissue, with no differences between males and females) after maternal exposure to 6 mg/kg RDX via gavage from gestational day (GD) 6 to PND 10. RDX was also detected in maternal milk (concentrations ranged from 3 to 5.7 µg/mL on PND 1 and from 0.7 to 3.1 µg/mL on PND 10).

C.1.3. Metabolism

The metabolism of RDX is not well characterized. No studies investigating the metabolism of RDX in humans were identified. Studies in animals indicate that RDX undergoes extensive metabolism, including denitration, ring cleavage, and generation of CO_2. Predominant metabolic pathways and major organs involved in RDX metabolism have not been identified, although results of in vitro studies suggest a role for CYP450.

RDX undergoes metabolism through processes that generate CO_2. In Sprague-Dawley rats administered a single 50 mg/kg gavage dose of [^{14}C]-RDX, 43% was recovered as exhaled [$^{14}CO_2$] after 4 days (Schneider et al., 1977). Similarly, approximately 30–50% of the radioactivity was recovered as exhaled [$^{14}CO_2$] in rats administered [^{14}C]-RDX in saturated drinking water or daily gavage for up to 3 months (Schneider et al., 1978). Metabolism of RDX to CO_2 was also observed in prairie voles following dietary exposure (average RDX dose per animal of 2.3 mg/kg-day) to

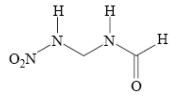
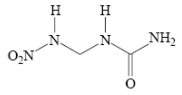
[^{14}C]-RDX incorporated plant materials for 5–7 days, with approximately 9% of the administered [^{14}C]-RDX dose eliminated as exhaled [$^{14}CO_2$] (Fellows et al., 2006).

Terminal metabolites of RDX have been identified in the urine of rats and swine, with very little urinary excretion of parent compound, indicating extensive metabolism of RDX. Following oral administration of a single 50 mg/kg gavage dose of [^{14}C]-RDX, 3.6% of the urinary radioactivity was identified as unmetabolized RDX (Schneider et al., 1977). Total urinary radiolabel accounted for about one-third of the administered label and unmetabolized RDX contributed 3–5% of total urinary radioactivity in rats exposed to [^{14}C]-RDX-saturated drinking water for 1 or 13 weeks (Schneider et al., 1978). Similar results were observed in Yucatan swine administered a single 45 mg/kg oral dose of [^{14}C]-RDX, with approximately 1–3.5% of the urinary radioactivity as parent RDX (Major et al., 2007). Urinary metabolites were not characterized in these studies (Schneider et al., 1978, 1977). However, Schneider et al. (1978) cited unpublished findings in their laboratory that, in addition to carbon dioxide, other one-carbon intermediates were produced, including bicarbonate and formic acid.

In the environment, the predominant breakdown products of RDX are methylene dinitramine and 4-nitro-2-diazbutanal (Sweeney et al., 2012b; Paquet et al., 2011). RDX metabolism in animals is less well understood. N-Nitroso RDX metabolites have been identified as derived through anaerobic metabolism (ATSDR, 2012; Pan et al., 2007b). Based on characterization of RDX metabolites in urine and plasma of miniature swine, metabolism of RDX appears to involve loss of nitro groups and ring cleavage (Musick et al., 2010; Major et al., 2007). The two principal urinary metabolites identified in miniature swine following a single oral dose of 43 or 45 mg/kg were 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diaza-butanamide (see Table C-2). Bhushan et al. (2003) suggested that the formation of the 4-nitro-2,4-diazabutanal metabolite occurred via denitration followed by hydroxylation and spontaneous hydrolytic decomposition resulting in ring cleavage and aldehyde formation. In the miniature swine gavage studies, only trace amounts of the nitrosamine RDX metabolites, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and 1-nitro-3,5-dinitroso-1,3,5-triazacyclohexane (DNX), were found in urine (Musick et al., 2010; Major et al., 2007). In plasma, most of the radioactivity existed as RDX, with trace levels of MNX, DNX, and 1,3,5-trinitroso-1,3,5-triazacyclohexane (TNX). The study authors suggested that the trace levels of MNX, DNX, and TNX in plasma may have been formed within the GI tract via sequential nitrogen reduction by intestinal bacteria (Major et al., 2007). The low levels of these compounds in urine and plasma were attributed to the nearly complete absorption of RDX from the GI tract, leaving little parent compound available for bacterial metabolism within the GI tract. In a study of female deer mice (*Peromyscus maniculatus*) fed diets containing RDX at concentrations of 12 and 120 mg/kg for 9 days, MNX and DNX were identified in the stomach, but TNX was not detected (Pan et al., 2007b). MNX and DNX were also measured in various organs of female B6C3F$_1$ mice provided RDX in feed at doses of 0.38–522 mg/kg; TNX was also detected in some organ compartments, but not in the liver. The authors concluded that RDX can be metabolized into its N-nitroso compounds

in mice, but did not identify a mechanism for the formation of the metabolites. Comparing RDX with MNX and TNX, RDX was the most potent compound at causing overt signs of toxicity (seizures and mortality) as determined through identification of the median lethal dose using the EPA up-and-down procedure in deer mice of varying ages (Smith et al., 2009; Rispin et al., 2002).

Table C-2. Principal urinary metabolites of RDX in miniature swine 24 hours after dosing with RDX

Sample origin	Metabolite name	Metabolite structure
Urine peak 1 M1	4-Nitro-2,4-diazabutanal	
Urine peak 2 M2	4-Nitro-2,4-diaza-butanamide	

Sources: Major et al. (2007); Musick et al. (2010)

Although the metabolic pathways and major tissues involved in RDX metabolism have not been identified, there is some evidence for the involvement of the liver and CYP450 enzymes. Comparison of hepatic radioactivity to liver concentrations of RDX after a single gavage dose to rats suggested the presence of RDX metabolites and a possible role for hepatic metabolism of RDX (Schneider et al., 1977). In vitro data indicated that CYP450 may be involved in the metabolism of RDX (Bhushan et al., 2003). Incubation of RDX with nicotinamide adenine dinucleotide phosphate (NADPH) and rabbit liver CYP450 2B4 under anaerobic conditions produced nitrite, 4-nitro-2,4-diazabutanal, formaldehyde, and ammonium ion (Bhushan et al., 2003). The reaction rate under aerobic conditions was approximately one-third of that observed under anaerobic conditions. Several CYP450 inhibitors (ellipticine, metyrapone, phenylhydrazine, 1-aminobenzo-triazole, and carbon monoxide) decreased the formation of RDX metabolites (55–82% inhibition), providing support for the role of CYP450 in RDX metabolism.

C.1.4. Excretion

The primary routes of elimination of absorbed RDX are excretion of RDX and metabolites in urine, and exhalation of CO_2 liberated from metabolism of RDX (Sweeney et al., 2012a; Musick et al., 2010; Krishnan et al., 2009; Major et al., 2007; Schneider et al., 1977). Tritium derived from administered [^{3}H]-RDX has been detected in mouse gall bladder contents, suggesting biliary secretion in this species (Guo et al., 1985); however, biliary secretion of RDX or metabolites has not been confirmed in other animal species. Studies conducted in the rat and swine suggest that metabolism is the dominant mechanism of elimination of absorbed RDX. In both species, metabolites dominated the carbon-14 distribution in urine of animals that received doses of

[^{14}C]-RDX, with RDX accounting for <5% of the urinary carbon-14 (Musick et al., 2010; Schneider et al., 1977).

Data on kinetics of elimination of absorbed RDX from blood are available from reports of accidental exposures of humans to RDX (Table C-3). Woody et al. (1986) estimated the elimination $t_{1/2}$ to be approximately 15 hours in a child who ingested approximately 85 mg of RDX per kg of body weight. The $t_{1/2}$ estimate was based on measured serum concentrations of RDX made between 24 and 120 hours following ingestion for RDX. Based on plasma RDX concentration data from five adults exposed to RDX (measurements made between 24 and 96 hours following exposure) (Özhan et al., 2003), a first-order elimination $t_{1/2}$ of 20–30 hours was derived (calculated for this review by fitting the serum RDX data to a first-order exponential function). It needs to be noted that it is not possible to draw reliable inferences from these values since they are based on accidental, acute exposures and, in particular, the data for the child are based on a single set of measurements for one individual.

Table C-3. Elimination $t_{1/2}$ values for RDX or radiolabeled RDX

Animal	Route	Dose (mg/kg)	Time[a]	$t_{1/2}$ (hrs)	Source
Human (child)	Oral	85[b]	24–120 hrs	15.0[c]	Woody et al. (1986)
Human (adult)	Oral	NA	24–96 hrs	21–29[c,d]	Özhan et al. (2003)
Rat	i.v.	5–6	0.5 min–6 hrs	10[b]	Schneider et al. (1977)
Rat	i.v.	0.8–1.0	30 min–10 hrs	4.6[c,d]	Krishnan et al. (2009)
Rat	Oral	1.53–2.07	1–10 hrs	6.9[c,d]	Krishnan et al. (2009)
Mouse	Oral	35, 60, 80	45 min–4 hrs	1.2[d]	Sweeney et al. (2012b)

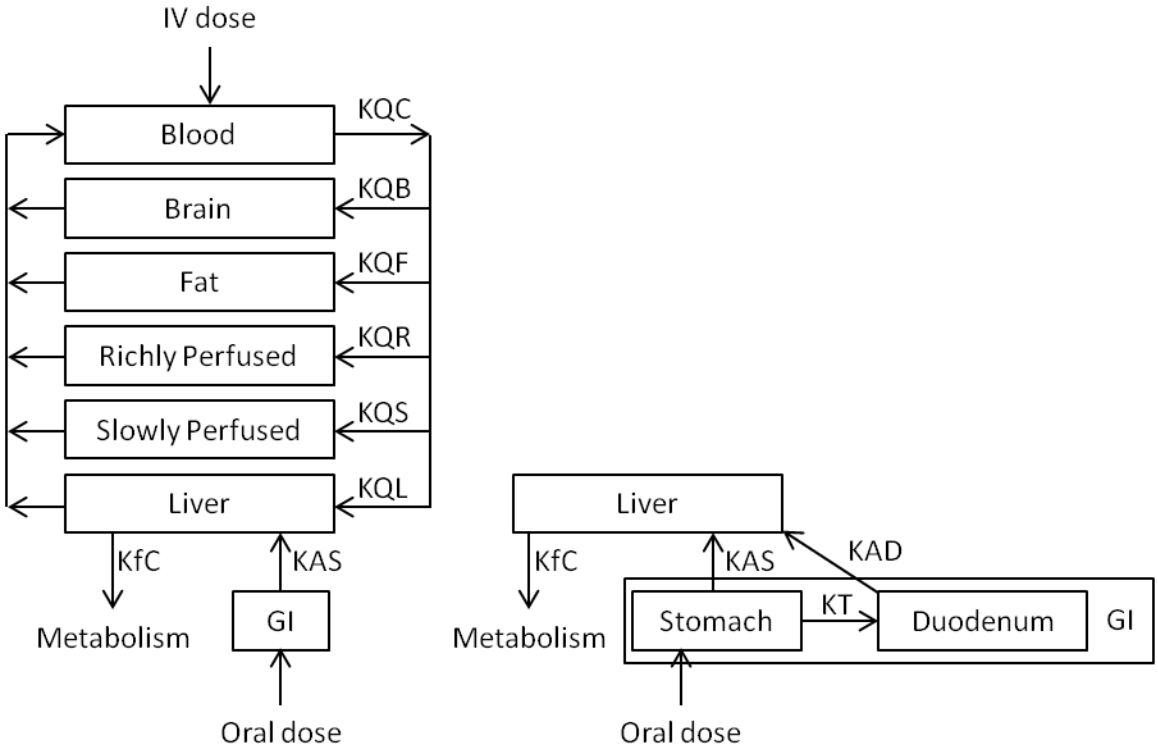
[a]Observation period following exposure on which the $t_{1/2}$ values were based.
[b]Reported estimate of dose based on blood kinetics.
[c]Value for blood RDX.
[d]Calculated for this review based on reported blood RDX concentrations.

The kinetics of elimination of absorbed RDX from blood has been evaluated in rats and mice. In rats elimination kinetics were biphasic (Krishnan et al., 2009; Guo et al., 1985; Schneider et al., 1977). As shown in Table C-3, estimated $t_{1/2}$ values for the terminal elimination phase in rats range from 5 to 10 hours (Krishnan et al., 2009; Schneider et al., 1977). Blood concentration time course measurements of RDX can be used to estimate an apparent metabolism and elimination of RDX from blood. The RDX blood concentrations reported in Sweeney et al. (2012b) after gavage dosing of 35, 60, and 80 mg/kg RDX found a consistent terminal elimination $t_{1/2}$ of approximately 1.2 hours. The elimination $t_{1/2}$ estimated for rats (Krishnan et al., 2009; Schneider et al., 1977) is as much as an order of magnitude longer than mice (Sweeney et al., 2012b).

C.1.5. Physiologically Based Pharmacokinetic (PBPK) Models

Overview of Available PBPK Models

A PBPK model to simulate the pharmacokinetics of RDX in rats was first developed by Krishnan et al. (2009) and improved upon 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 (Figure C-1). The model can simulate RDX exposures via the i.v. or oral route. Distribution of RDX to tissues is assumed to be flow-limited. Oral absorption is represented in this model as first-order uptake from the GI tract into the liver, with 100% of the dose absorbed. RDX is assumed to be cleared by first-order metabolism in the liver. However, there is no representation of the kinetics of any RDX metabolites. The acslX model code (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, Alabama) was obtained from the authors of Sweeney et al. (2012a).



Exposure to RDX is by the i.v. or oral route, and clearance occurs by metabolism in the liver. See Table C-4 for definitions of parameter abbreviations. The GI tract is represented as one compartment in Krishnan et al. (2009) (on the left) and two compartments in Sweeney et al. (2012a) (on the right).

Figure C-1. PBPK model structure for RDX in rats and humans.

The parameter values used in the Sweeney et al. (2012a) rat model are listed in Table C-4. The physiological model parameter values for cardiac output, tissue volumes, and blood perfusion of tissues were obtained from the literature (Timchalk et al., 2002; Brown et al., 1997). RDX tissue:blood partition coefficients for liver (PL), brain (PB), and richly perfused tissues (PR) were estimated with an algorithm that relates the measured n-octanol:water partition coefficient for RDX to reported compositions of water and lipids in specific rat tissues (Poulin and Theil, 2000; Poulin and Krishnan, 1995). Tissue:blood partition coefficients for fat (PF) and slowly perfused tissues (PS), as well as the metabolic rate constant (KfC), were simultaneously optimized to fit rat blood RDX concentrations following i.v. doses of 0.77 or 1.04 mg/kg RDX (Krishnan et al., 2009) producing values of 5.57, 0.15, and 2.6 $kg^{0.33}$/hour for PF, PS, and KfC, respectively. While the optimized value for PS is much smaller than that used by Krishnan et al. (2009) (1.0 $kg^{0.33}$/hour), the optimized values for PF and KfC were fairly similar to those used by Krishnan et al. (2009) (7.55, and 2.2 $kg^{0.33}$/hour). The rat model with these parameter values also had good agreement with blood RDX concentrations after a 5–6 mg/kg i.v. exposure (Schneider et al., 1977).

Table C-4. Parameter values used in the Sweeney et al. (2012a) and Sweeney et al. (2012b) PBPK models for RDX in rats, humans, and mice as reported by authors

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Body weight (BW; kg)	0.3	70	0.0206	Default values; study-specific values used if available
Cardiac output (KQC, L/hr/$kg^{0.74}$)	15	14	15	Timchalk et al. (2002); Brown et al. (1997)
Tissue volumes (fraction of BW)				
Liver (KVL)	0.04	0.026	0.04	Timchalk et al. (2002); Brown et al. (1997)
Brain (KVB)	0.012	0.02	0.012	Timchalk et al. (2002); Brown et al. (1997)
Fat (KVF)	0.07	0.21	0.07	Timchalk et al. (2002); Brown et al. (1997)
Richly perfused tissues (KVR)	0.04	0.052	0.04	Timchalk et al. (2002); Brown et al. (1997)
Blood (KVV)	0.06	0.079	0.06	Timchalk et al. (2002); Brown et al. (1997)
Slowly perfused tissues (KVS)	0.688	0.523	0.688	0.91 – (KVL + KVB + KVF + KVR + KVV)
Blood flows (fraction of cardiac output)				
Liver (KQL)	0.25	0.175	0.25	Timchalk et al. (2002); Brown et al. (1997)
Brain (KQB)	0.03	0.114	0.03	Timchalk et al. (2002); Brown et al. (1997)
Fat (KQF)	0.09	0.085	0.09	Timchalk et al. (2002); Brown et al. (1997)
Slowly perfused tissues (KQS)	0.2	0.2449	0.2	Timchalk et al. (2002); Brown et al. (1997)
Richly perfused tissues (KQR)	0.43	0.3811	0.43	1 – (KQL + KQB + KQF + KQS)

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Tissue:blood partition coefficients				
Liver (PL)	1.2	1.3	1.3	Krishnan et al. (2009)[a]
Brain (PB)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Richly perfused tissues (PR)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Fat:blood (PF)	5.57	5.57	5.57	Sweeney et al. (2012a)[b]
Slowly perfused tissues (PS)	0.15	0.15	0.15	Sweeney et al. (2012a)[b]
Metabolism				
First-order metabolic rate constant (KfC; $kg^{0.33}$/hr)	2.6	9.87 (child); 11.2 (adult)	102	Sweeney et al. (2012a)[b,c]; Sweeney et al. (2012b)[d]
GI absorption				
Dosing via gavage				
Absorption from compartment 1 (KAS, /hr)	0.83	0.033	0.51	Sweeney et al. (2012a); Sweeney et al. (2012b)[c,d,e]
Transfer from compartment 1 to compartment 2 (KT, /hr)	1.37	0	0	Sweeney et al. (2012a)[c,d]
Absorption from compartment 2 (KAD, /hr)	0.0258	0	0	Sweeney et al. (2012a)[c,d]
Dosing via capsule (KAS, /hr)	0.12	NA	NA	Sweeney et al. (2012a)[e]
"coarse" RDX formulation (KAS, /hr)	0.005	NA	NA	Sweeney et al. (2012a)[e]

[a]Predicted from n-octanol:water partition coefficient.
[b]Optimized from rat i.v. data.
[c]Optimized from human data of Özhan et al. (2003) and Woody et al. (1986).
[d]Optimized from mouse oral data.
[e]Optimized from rat oral data of Bannon et al. (2009a), Crouse et al. (2008), Krishnan et al. (2009), and Schneider et al. (1977).

Note: Parameter values used in the Sweeney et al. (2012a) and Sweeney et al. (2012b) PBPK models for RDX in rats, humans, and mice.

The GI tract oral absorption rate constant (KAS) was optimized to fit the time-course concentration data for rat oral dosing studies. The Krishnan et al. (2009) model used a one-compartment GI tract. KAS was fit to the RDX blood concentrations in Krishnan et al. (2009), and the model with this parameter value had good agreement with the blood RDX concentrations after 0.2 and 1.24 mg/kg oral exposures (Crouse et al., 2008). The value of KAS was adjusted to fit the RDX blood concentrations in the Schneider et al. (1977) study. Sweeney et al. (2012a) modified the GI tract description by adding a second GI compartment and corresponding oral absorption parameters (KAS, KAD, and KT) to fit the blood concentrations from Krishnan et al. (2009). For the other oral dosing studies, the two-compartment GI model did not improve the model fit to the data,

so KT was set equal to zero making the GI submodel equivalent to a one-compartment model. The value of KAS was adjusted separately to fit the oral studies with RDX in capsules (Bannon et al., 2009a; Crouse et al., 2008) and coarse-grain RDX in a saline slurry (Schneider et al., 1977).

The Sweeney et al. (2012a) model fits to blood and brain RDX concentrations for rats were mostly within a factor of 1.5 of the experimentally measured values indicating a tightly calibrated model.

Human RDX toxicokinetics were modeled with the same model structure as for rats. Values for the human physiological parameters such as tissue volumes and blood perfusion of tissues were obtained from the literature (Brown et al., 1997). Human absorption and metabolic clearance rate constants were optimized to fit observed RDX blood concentrations from a case study of ingestion by a 3-year-old boy (Woody et al., 1986), and a study where five soldiers were intentionally or accidentally exposed to RDX powder via inhalation or dermal contact (Özhan et al., 2003). The amounts of RDX ingested in both studies were unknown, so Sweeney et al. (2012a) estimated the dose amount by optimizing this parameter to fit the data (Table C-4). Sweeney et al. (2012a) initially simulated each individual soldier's blood level data separately. The resulting parameter values were similar, so data from the five soldiers were combined and the rate constants were re-estimated using the combined data. For comparison, the rat metabolic rate constant (KfC) was scaled to humans; the rat KfC (from fitting to in vivo data) was multiplied by the ratio of the human to rat metabolic rate constants measured in vitro and by the ratio of human to rat microsomal protein levels (Cao et al., 2008; Lipscomb and Poet, 2008). The scaling from rats yielded a human in vivo metabolic rate constant of 12.4 kg-$BW^{0.33}$/hour, which is similar to the values that Sweeney et al. (2012a) derived by fitting the combined Özhan et al. (2003) adult data (11.2 kg-$bw^{0.33}$/hour) and the Woody et al. (1986) child data (9.87 kg-$bw^{0.33}$/hour).

Mouse RDX toxicokinetics were also modeled by Sweeney et al. (2012b) using the same model structure as for rats. Values for the mouse physiological parameters such as tissue volumes and blood perfusion of tissues were assumed to be the same as the body weight normalized parameter values in the rat model. RDX tissue:blood partition coefficients for liver (PL), brain (PB), and richly perfused tissues (PR) were estimated with an algorithm that relates the measured n-octanol:water partition coefficient for RDX to reported compositions of water and lipids in specific mouse tissues (Poulin and Theil, 2000; Poulin and Krishnan, 1995). The KfC and KAS were optimized to fit measured mouse RDX blood concentrations (Sweeney et al., 2012b). The KfC value estimated for the mouse (102 $kg^{0.33}$/hour) is much higher than those estimated for rats and humans (2.6 and 11.2 $kg^{0.33}$/hour, respectively); however, the KAS value (0.51/hour) fit to mouse data is similar to the value (0.83/hour) used in the RDX rat model for gavage in water. The Sweeney et al. (2012b) model predictions of blood RDX concentrations were in good agreement with the experimental mouse gavage data reported in the same study.

The above PBPK model was evaluated and subsequently modified by EPA for use in dose-response modeling in this assessment. This is detailed in the following section.

PBPK Model Evaluation and Further Development of the Sweeney et al. (2012a) and Sweeney et al. (2012b) Models

EPA evaluated and performed a quality control check of the PBPK models for RDX in rats, humans, and mice published by Sweeney and colleagues (Sweeney et al., 2012a; Sweeney et al., 2012b). The conclusions from these analyses are summarized below and then discussed in more detail:

1) The model code and the parameter values matched the published reports. Minor discrepancies in physiological parameters (KVR and KQS) were identified and updated in the model by EPA.

2) The absorption of RDX from the GI tract did not use a consistent structure; for gavage doses, the model used a two-compartment GI submodel and for other oral exposures (e.g., gelatin capsule), the model used a one-compartment GI submodel. The model was revised to have a one-compartment GI submodel to simulate all oral exposures with a consistent set of absorption parameters for each dosage formulation of administered RDX.

3) Additional oral rat data were identified from single-dose studies (MacPhail et al., 1985; Schneider et al., 1977) and subchronic studies (Schneider et al., 1978) and were used for model calibration as well as for independent comparison against model predictions.

4) In addition to the sensitivity analysis conducted by Sweeney et al. (2012b) on the mouse model, a sensitivity analysis in the rat and human models was performed.

5) The Sweeney et al. (2012b) mouse model used the same physiological parameters scaled to body weight as the rat model. This mouse model was revised to use mouse-specific physiological parameters.

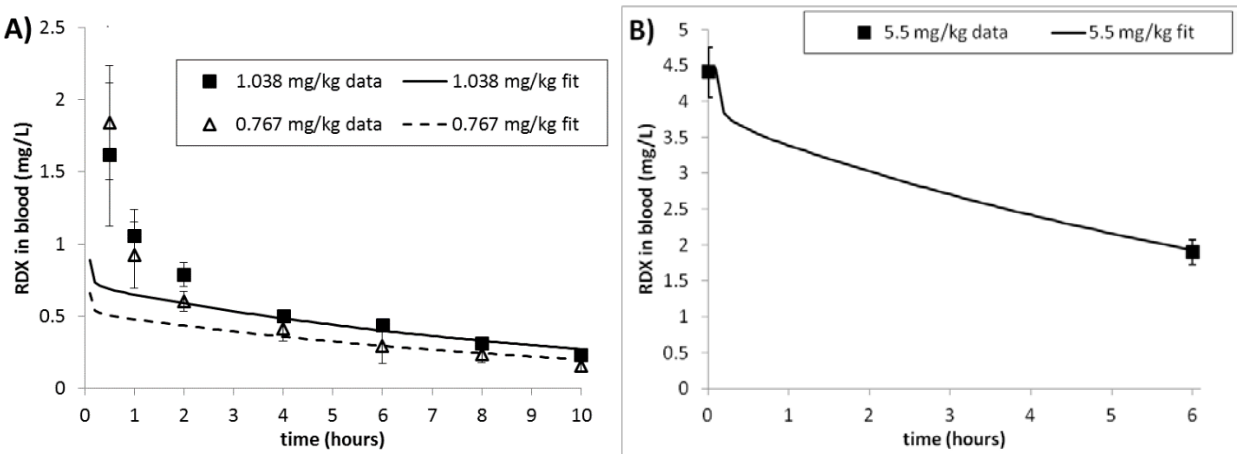
The Sweeney et al. (2012a) model for rats was modified by changing the oral absorption rate constants (as discussed below) and the partition coefficients for the fat and slowly perfused tissues (PF and PS) as shown in Table C-5. The partition coefficients for the fat and slowly perfused tissues were set to the values calculated by Krishnan et al. (2009) relating the measured n-octanol: water partition coefficient for RDX to reported compositions of water and lipids in those tissues. The fits to RDX blood time course data after i.v. exposure (Figure C-2) are slightly worse than the Sweeney et al. (2012a) rat model because the Sweeney et al. (2012a) rat model optimized the fat:blood and slowly perfused tissue partition coefficients to fit the data.

Table C-5. Parameters values used in the EPA application of the rat, human, and mouse models

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Body weight (BW; kg)	0.3	70	0.0206	Default values shown; study-specific values used if available
Cardiac output (KQC; L/hr/kg$^{0.74}$)	15	14	15	Timchalk et al. (2002); Brown et al. (1997)

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Tissue volumes (fraction of BW)				
Liver (KVL)	0.04	0.026	0.055	Timchalk et al. (2002); Brown et al. (1997)
Brain (KVB)	0.012	0.02	0.017	Timchalk et al. (2002); Brown et al. (1997)
Fat (KVF)	0.07	0.21	0.07	Timchalk et al. (2002); Brown et al. (1997)
Richly perfused tissues (KVR)	0.04	0.054	0.071	Timchalk et al. (2002); Brown et al. (1997)
Blood (KVV)	0.06	0.079	0.049	Timchalk et al. (2002); Brown et al. (1997)
Slowly perfused tissues (KVS)	0.688	0.523	0.648	0.91 − (KVL + KVB + KVF + KVR + KVV)
Blood flows (fraction of cardiac output)				
Liver (KQL)	0.25	0.175	0.25	Timchalk et al. (2002); Brown et al. (1997)
Brain (KQB)	0.03	0.114	0.03	Timchalk et al. (2002); Brown et al. (1997)
Fat (KQF)	0.09	0.085	0.09	Timchalk et al. (2002); Brown et al. (1997)
Slowly perfused tissues (KQS)	0.2	0.249	0.2	Timchalk et al. (2002); Brown et al. (1997)
Richly perfused tissues (KQR)	0.43	0.377	0.43	1 − (KQL + KQB + KQF + KQS)
Tissue:blood partition coefficients and metabolism				
Liver (PL)	1.2	1.3	1.3	Krishnan et al. (2009)[a]
Brain (PB)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Richly perfused tissues (PR)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Fat:blood PC (PF)	7.55	7.55	7.55	Krishnan et al. (2009)[a]
Slowly perfused tissues (PS)	1.0	1.0	0.9	Krishnan et al. (2009)[a]
First-order metabolic rate constant (KfC; $kg^{0.33}$/hr)	2.6	9.87 (small boy); 11.2 (soldiers)	77	Sweeney et al. (2012a)[b,c]; Sweeney et al. (2012b)[d]
Absorption				
Absorption from GI to liver (KAS; /hr)	Table C-6	1.75	0.6	Fit to rat, human, and mouse oral data
Absorption from lung to blood (Klung; /hr)		0.75		Fit to human data

[a]Predicted from n-octanol:water partition coefficient.
[b]Optimized from rat i.v. data.
[c]Optimized from human data of Özhan et al. (2003) and Woody et al. (1986).
[d]Optimized from mouse oral data, and differs from that obtained by Sweeney et al. (2012b).



A) data from Krishnan et al. (2009) (0.4 kg rats) and B) data from Schneider et al. (1977) (simulation of 0.25 kg rats and 5.5 mg/kg dose for 0.2–0.25 kg rats and 5–6 mg/kg dose).

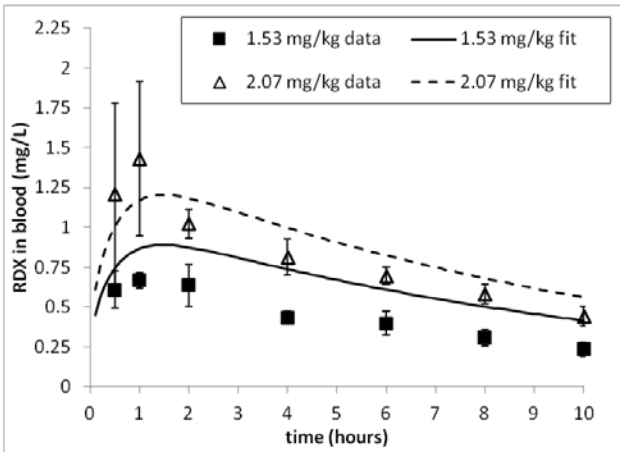
Figure C-2. EPA rat PBPK model predictions fitted to observed RDX blood concentrations in male and female Sprague-Dawley rats following i.v. exposure.

Absorption of RDX from the GI Tract

As discussed above in the oral absorption section under toxicokinetics (Section C.1.1), the rate of oral absorption depends on the physical form of RDX. This was demonstrated by comparing the Schneider et al. (1977) studies, which used gavage doses of 100 mg/kg of course, granular RDX and 50 mg/kg finely powdered RDX, and observing that the 50 mg/kg finely powdered RDX had a higher peak plasma level. These results are likely explained by the smaller surface area to mass ratio of the coarse-grain RDX leading to slower dissolution and absorption.

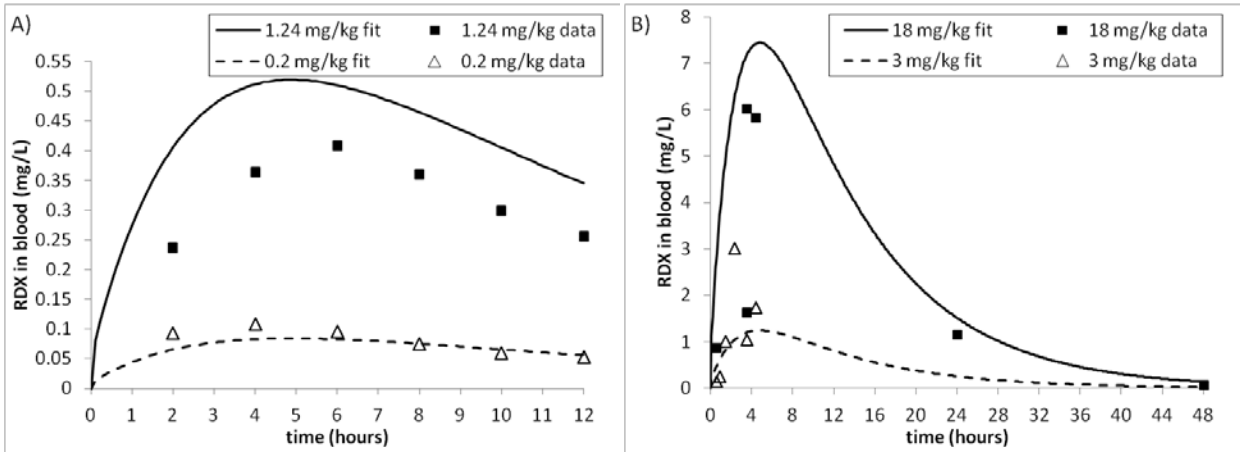
To follow the rule of model parsimony (i.e., use no more parameters than needed for the best fit to all of the data), oral absorption was modeled with a one-compartment GI tract submodel for all simulations. To account for the differences in absorption due to the physical form of RDX, separate rate constants for RDX oral absorption were optimized to fit measured blood concentrations of RDX according to the type of dosing formulation; the model fits obtained with the EPA's revised parameters for rats are shown in Figures C-3 to C-5. The oral dosing formulations were grouped into four categories: RDX dissolved in water, RDX in capsules, fine-grain RDX, and coarse-grain RDX. The absorption rate constant for RDX dissolved in water was optimized to the data in the Krishnan et al. (2009) study (Figure C-3). The absorption rate constant for RDX in capsules was optimized to the data in the Crouse et al. (2008) and Bannon et al. (2009a) studies (Figure C-4). The absorption rate constant for fine-grain RDX was optimized to the data described below (*Additional RDX Time-Course Data)* in the MacPhail et al. (1985) and Schneider et al. (1977) studies (Figure C-7). The Schneider et al. (1977) study was used to estimate the absorption rate constants for coarse-grain RDX (Figure C-5; as represented by the fit to the data obtained by the solid curve at 100% bioavailability). Overall, the fits of the EPA revised model to the blood time-

course data of these studies are similar to the fits of the Sweeney et al. (2012a) rat model. The fits to RDX brain time course data after oral exposure to RDX in capsules (Figure C-6A) are similar to the fits of the Sweeney et al. (2012a) rat model. The absorption rate constants for each dosing formulation are listed in Table C-6.



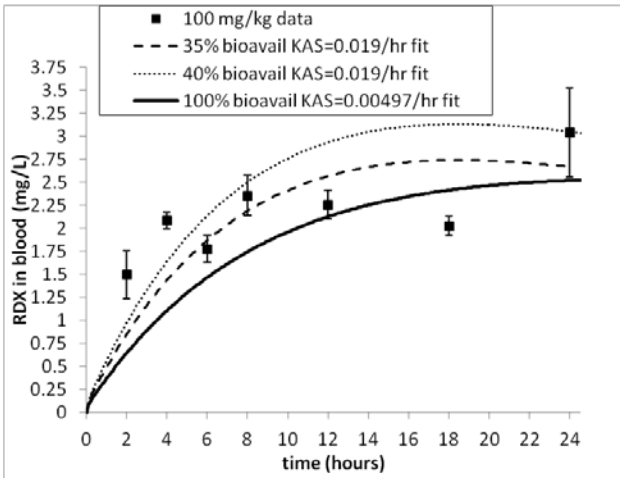
Male and female Sprague-Dawley rats (0.4 kg) were dosed by gavage (Krishnan et al., 2009).

Figure C-3. EPA rat PBPK model predictions fitted to observed RDX blood concentrations following oral exposure to RDX dissolved in water.



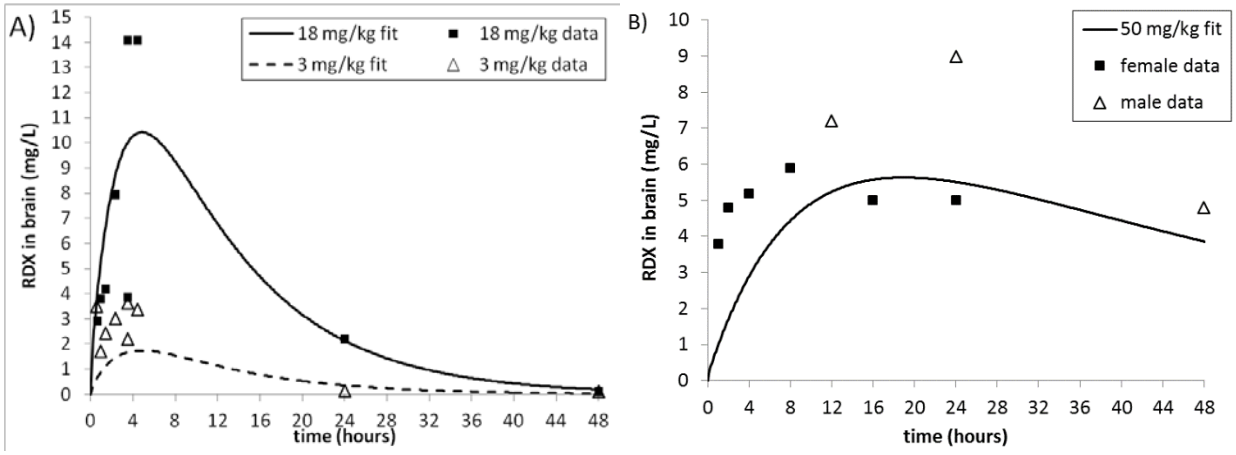
The ingested RDX doses were: A) 0.2 and 1.24 mg/kg RDX in male Sprague-Dawley rats (0.4 kg, data from Crouse et al. (2008)) and B) 3 and 18 mg/kg RDX in male and female Sprague-Dawley rats (0.35 kg, data from Bannon et al. (2009a)) for KAS = 0.35/hour.

Figure C-4. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to RDX in dry capsules.



Symbols denote observed RDX blood concentrations measured in male Sprague-Dawley rats (0.225 kg) resulting from oral doses of 100 mg/kg RDX (Schneider et al., 1977). The KAS fit to these data assuming 100% bioavailability resulted in the same estimate (KAS = 0.00497/hour) as obtained by Sweeney et al. (2012a). Alternatively, for KAS fixed at the value fit to fine-grain RDX in a saline slurry (KAS = 0.019/hour fit to data from Schneider et al. (1977) and MacPhail et al. (1985); Figure C-7), the estimate of oral bioavailability fit to the RDX blood concentrations was 35%. A bioavailability of 40% and KAS = 0.019/hour is also shown for comparison.

Figure C-5. Effect of varying oral absorption parameters on EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to coarse-grain RDX.



A) 3 and 18 mg/kg RDX in dry capsules (0.35 kg male and female rat data from Bannon et al. (2009a); best fit KAS = 0.35/hour. B) 50 mg/kg fine-grain RDX in a saline slurry (0.25 kg male and female rats data from MacPhail et al. (1985); best fit KAS = 0.019/hour.

Figure C-6. EPA rat model predictions fitted to observed RDX brain tissue concentrations following oral exposure to RDX.

Table C-6. Doses, dosing formulations, and absorption rate constants in animal and human studies

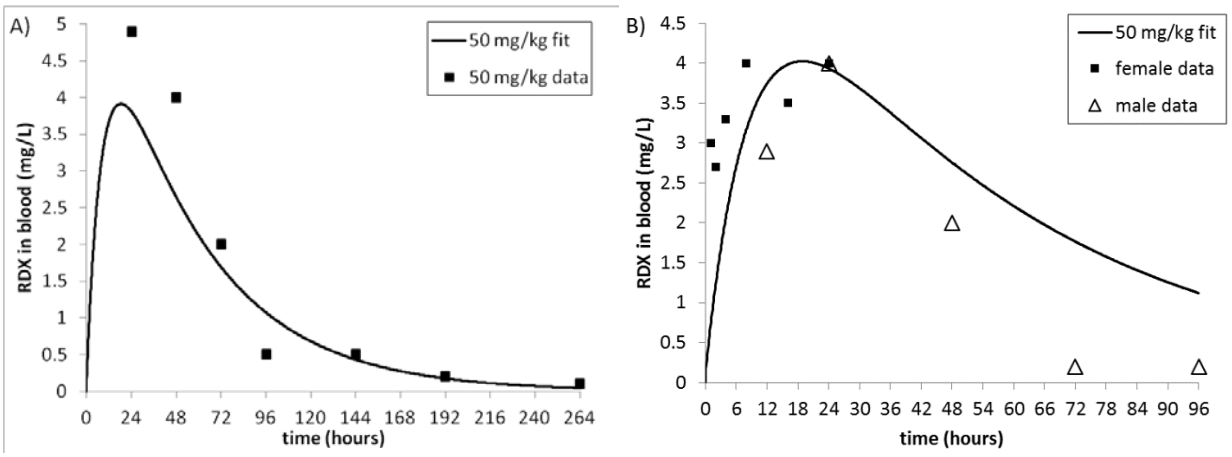
Formulation	Study	Dose	Estimated KA (/hr)
RDX dissolved in water	Krishnan et al. (2009)	1.53, 2.07 mg/kg, single gavage	1.75
	Schneider et al. (1978)	~5–8 mg/kg-d, drinking water 90 d	
Dry RDX in capsules[a]	Crouse et al. (2008)	0.2, 1.24 mg/kg, single dose	0.35
	Bannon et al. (2009a)	3, 18 mg/kg, single dose	
Fine-grain RDX in saline slurry	Schneider et al. (1977)	50 mg/kg, single gavage	0.19
	MacPhail et al. (1985)[b]	50 mg/kg, single gavage	
Coarse-grain RDX in saline slurry	Schneider et al. (1977)	100 mg/kg, single gavage	0.00497

[a]Capsules were filled with dry RDX from stock solution of acetone, and acetone was evaporated off.
[b]RDX particle size was ≤66 µm in diameter suspended in a 2% solution of carboxymethylcellulose.

An alternative to varying the KAS for each RDX formulation would be to vary the oral bioavailability, in effect modifying the administered exposure concentration. Therefore, the sensitivity of the model fit to variations in oral bioavailability was examined in Figure C-5 and an analysis of model sensitivity to oral bioavailability was conducted as discussed further in the section, *Sensitivity Analysis of the Rat PBPK Model*.

Additional RDX Time-Course Data

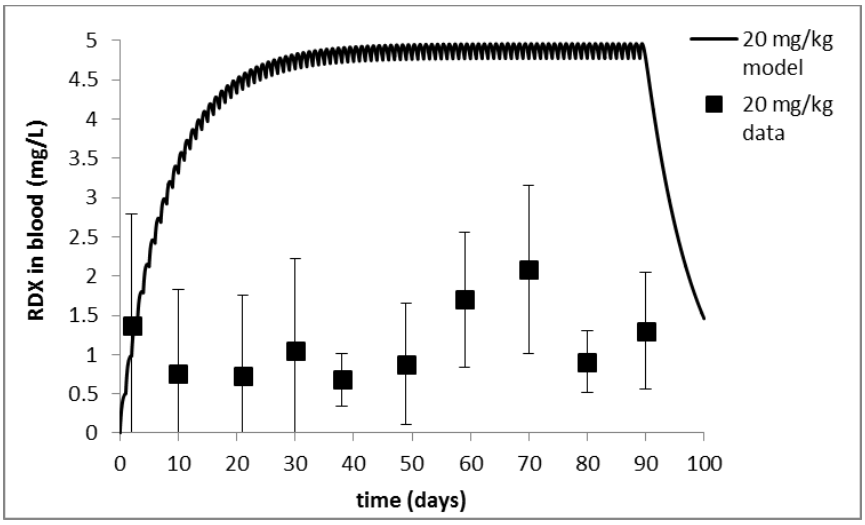
The EPA revised models were simultaneously fitted against additional RDX time-course data (not used in the original Sweeney et al. (2012a) model calibration). These data came from (1) two studies in which animals received oral doses of fine-grain RDX (MacPhail et al., 1985; Schneider et al., 1977) (Figure C-7) and (2) RDX brain time-course data from a study in which animals received oral doses of fine-grain RDX (MacPhail et al., 1985) (Figure C-6B). Overall, the calibrated EPA rat model predictions are within a factor of 1.5 of the measured values from different data sets, and are therefore likely to provide a more robust estimated parameter.



Oral doses of 50 mg/kg RDX were administered to: A) male Sprague-Dawley rats (0.225 kg) (Schneider et al., 1977) and B) male and female Sprague-Dawley rats (0.25 kg) data (MacPhail et al., 1985). Best fit KAS = 0.019/hour.

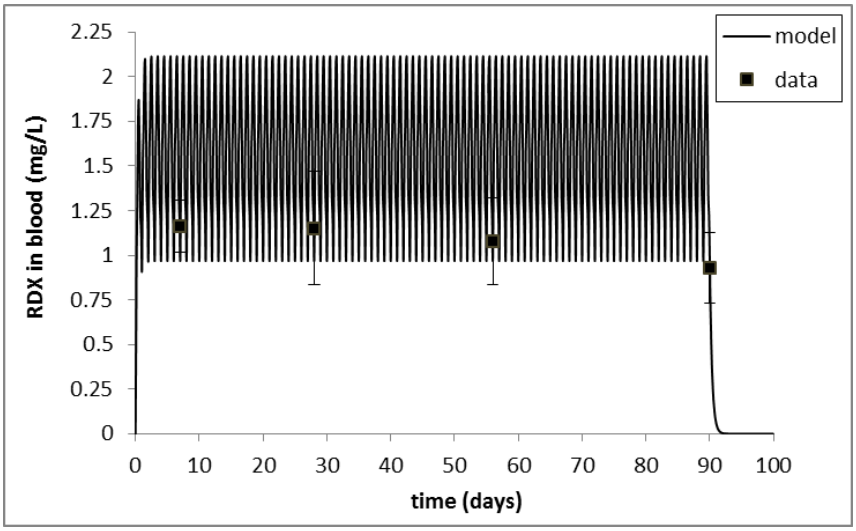
Figure C-7. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to fine-grain RDX in a saline slurry.

Following calibration, the EPA model was further tested by comparison with results from two other subchronic oral studies in male and female rats (Schneider et al., 1978). These were a gavage study where 20 mg/kg RDX was administered in saline slurry and a drinking water study where rats were provided with RDX-saturated drinking water (50–70 µg/mL) ad libitum for which the study authors estimated a daily dose between 5 and 8 mg RDX/kg body weight. It is striking that the observed RDX blood concentrations in the gavage study (Figure C-8, symbols) were virtually the same, or only slightly elevated, as compared to the blood concentrations reported in the drinking water exposures, with an approximately threefold lower daily administered dose in the drinking water study (Figure C-9, symbols). This is counter to the expectation that higher doses cause higher blood levels and is discussed further below.



Model fits and mean observed RDX blood concentrations resulting from daily gavage doses of 20 mg/kg RDX for 90 days to male and female Sprague-Dawley rats (0.225 kg). The RDX in saline slurry was assumed to be coarse-grained with an oral absorption rate constant KAS = 0.00497/hour.

Figure C-8. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic gavage study.



Model fits and mean observed RDX blood concentrations resulting from a daily estimated dose of 6.5 mg RDX/kg-day for 90 days to male and female Sprague-Dawley rats (0.225 kg). The large peak to trough change in the simulation results from model representation of the daily oral ingestion of drinking water primarily during the waking state. The oral absorption rate constant for RDX dissolved in water was used (KAS = 1.75/hour).

Figure C-9. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic drinking water study.

EPA's modified PBPK model was set up to simulate drinking water exposures with a noncontinuous sipping pattern based on Spiteri (1982), which assumed 80% of the consumption to occur episodically at night when the rats were awake[3]. The model predicts blood concentrations to increase in proportion to the total dose; for the gavage study, the model predictions yielded an RDX blood concentration approximately threefold higher than the reported mean blood concentrations (Figure C-8), while for the subchronic drinking water study, the model fit the data reasonably well (Figure C-9).

It is possible that multiple mechanisms such as elimination of unabsorbed RDX or metabolic induction may explain why the observed RDX blood concentrations did not increase in proportion to the higher administered dose in the gavage studies compared to the drinking water study. Elimination of unmetabolized RDX may be an insignificant factor in the single-dose studies used for calibration of the absorption constant for the RDX in saline slurry, but for repeated, higher doses this elimination route could be significant. Schneider et al. (1978) found similar RDX concentrations in the feces of rats in the gavage and drinking water studies (3.1 ± 2.0 and 2.7 ± 1.3 µg RDX per g dry weight feces, respectively). The total recovery of radioactivity in feces was also similar in the gavage study (4.8 ± 0.8%, week 1 only) and drinking water study (4.4 ± 0.6%, measured over the course of the study). Thus, the difference in fecal elimination for the two routes does not appear significant.

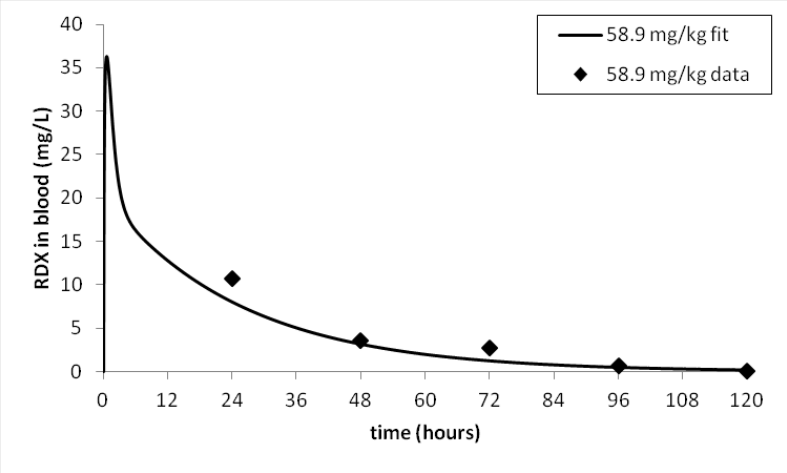
It is also possible that metabolic induction occurred during the repeated dosing of RDX in the gavage study leading to the lower observed RDX blood concentrations. The reasonably good fits of the model to the drinking water data set demonstrated achievement of regular periodic levels, and indicate a lack or much lower extent of metabolic induction over time from those repeated doses, possibly because the dose rate was lower: 5–8 versus 20 mg/kg-day in the gavage study. Overall, the reasonable agreement of the modified EPA RDX rat model with the subchronic drinking water data support the use of the model in estimating and extrapolating blood levels following chronic exposure at or below this exposure range (5–8 mg/kg-day), particularly in drinking water.

Simulating Exposures in Humans

The Sweeney et al. (2012a) model for humans was modified in the same ways as the rats, by changing the partition coefficients for the fat and slowly perfused tissues (PF and PS) as shown in Table C-5 and fitting the rate constants for oral absorption and metabolism to RDX blood concentration data. In the studies of humans with measured RDX blood concentrations by Woody et al. (1986) and Özhan et al. (2003), the RDX doses were unknown and the doses were therefore also optimized to fit the data. The model predictions for the Woody et al. (1986) data using the best fit values of dose = 58.9 mg/kg, KAS = 1.75/hour, and KfC = 9.87 $kg^{0.33}$/hour are shown in

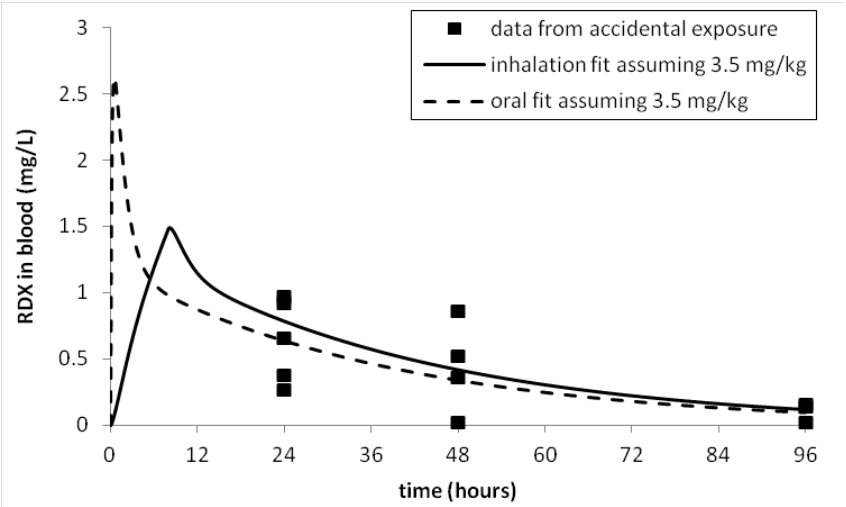
[3]A constant drinking water ingestion rate interspaced between episodes of no ingestion was assumed. Each 12-hour awake period consisted of eight cycles that alternated between 1.5-hour cycles of frequent sipping (continuous ingestion) and zero ingestion for 45 minutes each. Each 12-hour sleeping period consisted of four cycles with regular sipping periods of 30 minutes followed by 2.5 hours of no ingestion.

Figure C-10. The model predictions for the Özhan et al. (2003) data using the best fit values of an oral dose = 3.5 mg/kg, KAS = 1.75/hour, and KfC = 9.87 $kg^{0.33}$/hour are shown in Figure C-11.



The best fit values were KAS = 1.75/hour, dose = 58.9 mg/kg, and KfC = 9.87 $kg^{0.33}$/hour.

Figure C-10. EPA human model predictions fitted to observed RDX blood concentrations resulting from an accidental ingestion of RDX by a 14.5-kg boy (Woody et al., 1986).



For an assumed oral exposure, the best fit values were KAS = 1.75/hour, dose = 3.5 mg/kg, and KfC = 9.87 $kg^{0.33}$/hour. For the same 3.5 mg/kg dose and metabolism rate constant, an inhalation exposure found a best fit value for Klung of 0.75/hour.

Figure C-11. EPA human model predictions fitted to observed RDX blood concentrations resulting from accidental exposure to adults assumed to be 70 kg (Özhan et al., 2003).

EPA's calibration of the model differed in another important respect from that carried out by Sweeney et al. (2012a). As previously mentioned, Sweeney et al. (2012a) simulated the soldiers' exposure from the Özhan et al. (2003) study as an oral exposure, although the study report states that the exposure was via inhalation and dermal routes. An inhalation or dermal exposure could change the amount of RDX reaching the blood compared with an oral exposure due to first-pass metabolism in the liver after oral absorption. Dermal absorption was not considered by EPA to be a significant route of RDX exposure and was therefore not modeled. This decision is supported by a study that used excised human skin and reported that only 5.7% of the applied dose was absorbed into the skin by 24 hours post dosing (Reddy et al., 2008). The model was modified to simulate an inhalation exposure and compared with the data from Özhan et al. (2003). There are insufficient data on blood:air partitioning to modify the Sweeney et al. (2012a) model with a lung compartment; therefore, inhalation exposure was modeled in an approximate manner as a direct input to the blood with an optimized absorption rate to represent absorption from air containing RDX into the blood. The soldiers' inhalation exposure was simulated as a continuous 8-hour exposure (i.e., assuming that the soldiers were exposed occupationally during an 8-hour workday), and for the same dose of 3.5 mg RDX/kg that was estimated by Sweeney et al. (2012a). The model assumed that 100% of the inhaled dose was absorbed and that the absorption rate constant was optimized to fit the measured blood concentrations of RDX. The model predictions were in good agreement with the RDX blood concentrations reported by Özhan et al. (2003) as shown in Figure C-11.

Sensitivity Analysis of the Rat and Human PBPK Models

A sensitivity analysis was performed to see how each model parameter affects the model output. A sensitivity coefficient, defined as the change in a specified dose metric due to a 1% increase in the value of a parameter, was calculated for each parameter in the rat and human models. This analysis was carried out for both short-term (24 hours following a single oral dose of 1.5 mg/kg RDX) and longer-term (90 days of repeated oral dosing with 1.5 mg/kg RDX) exposures for the dose-metric of blood AUC. Parameters with sensitivity coefficients >0.1 in absolute value (i.e., considered sensitive) are presented in Table C-7. For the blood AUC dose-metric, the only sensitive RDX-specific parameter is the KfC. This sensitivity is likely because bioavailability was assumed to be 100% and metabolism is the only route of elimination in the model. These assumptions mean that all administered RDX will be absorbed and metabolized; in other words, the blood AUC is proportional to the dose and inversely proportional to the metabolic clearance rate constant. For the parameter values in this model, the rate of metabolism is relatively slow compared to the transport of RDX between other tissues and the site of metabolism in the liver, so that the blood AUC is not sensitive to parameters that impact transport such as KQC and KQL. Because the metabolic clearance rate constant is scaled to body weight and by liver volume, the blood AUC is also sensitive to these parameters. The sensitivity analysis by Sweeney et al. (2012b)

for the AUC of RDX in the liver found the model was sensitive to the liver:blood partition coefficient (PL) in addition to the same parameters (KfC, KVL, and BW) found for the blood AUC.

Table C-7. Sensitivity coefficients for rat and human RDX PBPK models

Parameter	Rat sensitivity coefficient	Human sensitivity coefficient
Fractional liver volume (KVL)	−1	−1
Body weight (BW)	0.3	0.3
Metabolic rate constant (KfC)	−1	−1

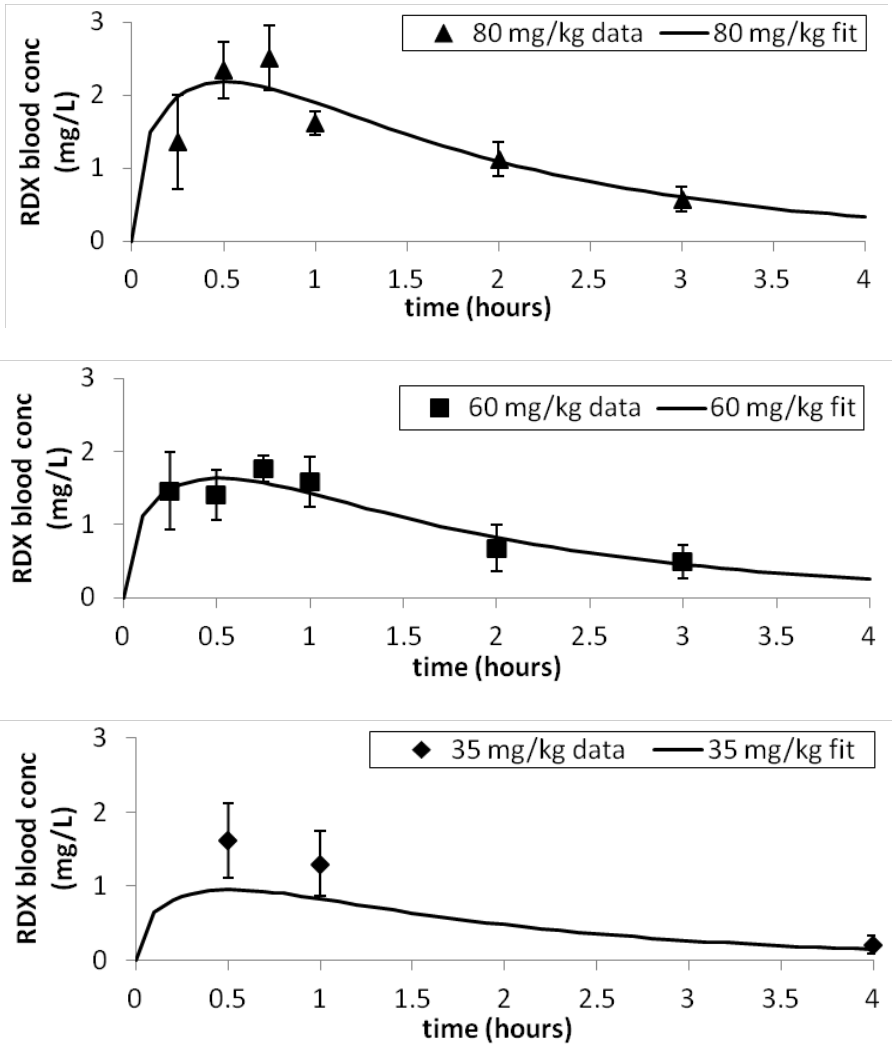
Parameters with sensitivity coefficients <0.1 in absolute value are considered not sensitive, and are listed below:

- cardiac output (KQC);
- fractional blood flow to all tissues (liver, KQL; fat, KQF; slowly perfused tissues, KQS; brain, KQB)
- fractional tissue volume of fat (KVF), brain (KVB), and blood volume (KVV)
- blood partition coefficients to all tissues (liver, PL; fat, PF; rapidly perfused, PR; slowly perfused, PS; brain, PB)
- absorption rates from GI (KAS, KT, KAD)

The model is also very sensitive to oral bioavailability, with a sensitivity coefficient of 0.8 in the case of the rat model. As discussed above in the oral absorption section of toxicokinetics (Section C.1.1), estimates of the bioavailability of RDX range from 50 to 87% or greater and may depend upon the physical form of RDX (Krishnan et al., 2009; Schneider et al., 1978, 1977). However, as seen in Figure C-5, it was not possible to identify the bioavailability and the absorption rate (KAS) as separate parameters by fitting to the available RDX blood concentration time course. Introducing oral bioavailability as an additional unknown parameter and recalibrating the model did not appear to provide an advantage. Therefore, 100% bioavailability was assumed in the model and was acknowledged as an uncertainty.

Simulating Exposures in Mice

Physiological parameters specific to mice were obtained from the literature (Brown et al., 1997) and are shown in Table C-5. The partition coefficients calculated for mice by Sweeney et al. (2012b) were used, and include the liver, brain, and richly perfused tissues. The partition coefficients for the fat and slowly perfused tissues from the Sweeney et al. (2012b) mouse model were not used because they were estimated via optimization of fits to rat i.v. data. Instead, the partition coefficient for fat tissues was set equal to the value calculated by Krishnan et al. (2009) for rat fat tissue, 7.55. The partition coefficient for slowly perfused tissues (0.9) was calculated for mouse tissues using the same methodology as Krishnan et al. (2009). The rate constants for oral absorption and metabolism were optimized to fit the data from Sweeney et al. (2012b) for mouse blood RDX concentrations. The model predictions were in good agreement with the RDX blood concentrations reported by Sweeney et al. (2012b), as shown in Figure C-12.



Model fits and mean and standard deviation of observed RDX blood concentrations in female B6C3F$_1$ mice (0.0205 kg) for doses of 35, 60, and 80 mg/kg with KAS = 0.6/hour and KfC = 77 kg$^{0.33}$/hour. Experimental data from Sweeney et al. (2012b).

Figure C-12. Comparison of EPA mouse PBPK model predictions with data from oral exposure to RDX dissolved in water.

The mouse RDX blood concentrations reported by Sweeney et al. (2012b), as shown in Figure C-12 were evaluated with a non-compartmental analysis and compared with the rat data. The estimate of the area under the curve for blood concentration versus time from the time of dosing to the time RDX is completely eliminated (AUC_{total}) was calculated with a linear trapezoidal sum plus an extrapolation of the blood concentration at the last time point divided by the terminal elimination rate constant as shown in the following equation:

AUC_{total} = Σ(Δblood concentrations) Δt/2 + blood concentration at last time point/K_{el}

where Δblood concentrations are the successive blood concentrations, Δt is the time between measured concentrations, and K_{el} is the terminal elimination rate constant (calculated from the slope of the linear regression line to the log of blood concentrations)

For the mouse data from Sweeney et al. (2012b) for the doses of 35, 60, and 80 mg/kg, the results of the AUC_{total} calculation are 3.35, 3.70, and 4.75 mg/L hour; normalized to the administered dose, these are 0.096, 0.062, and 0.059 mg/L hour per mg/kg. For the blood concentrations measured in rats in the Krishnan et al. (2009) study (Figure C-3), the animals received a single oral (gavage) dose of RDX dissolved in water similar to the Sweeney et al. (2012b) study. The Krishnan et al. (2009) study used doses of 1.53 and 2.07 mg/kg and the results of the AUC_{total} calculation are 6.1 and 11.9 mg/L hour. Including the extrapolation of the blood concentration from the last time point with the terminal elimination rate constant, K_{el} had a major contribution to the AUC_{total} (approximately one-third), which adds uncertainty to the result, so the AUC_{total} was also calculated without this term and the results are 4.1 and 7.5 mg/L hour. The AUC_{total} values normalized to the administered doses are 4.0 and 5.8 mg/L hour per mg/kg (including the extrapolation from the last time point) or 2.7 and 3.6 mg/L hour per mg/kg (excluding the extrapolation from the last time point). Overall, the AUC_{total} normalized to the administered doses for the rat are of the order 10–100 times greater than for the mouse. This non-compartmental analysis of the data is independent of the PBPK modeling and shows the extent of the toxicokinetic differences for RDX between the mouse and rat.

The only additional information on RDX metabolism in the mouse comes from a study by Pan et al. (2013). Pan et al. (2013) measured nitrosamine RDX metabolites of RDX (MNX, DNX, and TNX, the latter representing a minor metabolic pathway) in mice at the end of a 28-day exposure to RDX in feed (ad libitum). These measurements were a single time point without controlling the time between the last RDX ingestion and measurement, and were therefore judged not to be sufficient for use in parameterizing a PBPK model of the nitrosamine metabolites.

Rat to Human Extrapolations

The rat and human PBPK models as described above were applied to derive human equivalent doses (HEDs) for candidate points of departure (PODs) for endpoints selected from rat bioassays. The rat and human PBPK models were used to estimate two dose metrics—the AUC and the peak concentration (C_{max}) for RDX concentration in arterial blood. The relationships between administered dose and both internal metrics (AUC and C_{max}) were evaluated with the rat PBPK model over the range of 1 µg/kg-day to 100 mg/kg-day and with the human PBPK model over the range of 0.05 µg/kg-day to 200 mg/kg-day, ranges that encompass the PODs. The times to reach steady state for the dose metrics were shorter than the duration of the toxicity studies, so the

steady state values were considered representative of the study and were used. To calculate steady-state values for daily exposure, the simulations were run until the daily average had a <1% change between consecutive days. For both the rat and human PBPK models, both dose metrics correlated linearly with the administered dose. For rats dosed via gavage, the slope of administered dose versus AUC was 6.800 mg/L-day / mg/kg-day and that for C_{max} was 0.4718 mg/L / mg/kg-day. For a continuous dose, the slope of dose versus AUC was the same (6.800 mg/L-day / mg/kg-day) and for C_{max} was 0.3951 mg/L / mg/kg-day. For humans, assuming a drinking water dose sipping pattern, the slope of administered dose versus AUC was 13.95 mg/L-day / mg/kg-day and that for C_{max} was 0.7316 mg/L / mg/kg-day. Given this linearity in internal metrics and assuming that equal internal metrics in rats and humans are associated with the same degree of response, the HEDs could then be directly determined by multiplying the lower bound on the benchmark dose (BMDL) in rats by the ratio of these slopes. For a gavage dose in rats converted to a human drinking water dose, the ratio for AUC was 6.800 / 13.95 = 0.487 and C_{max} was 0.4718 / 0.7316 = 0.645. For a continuous dose in rats converted to a human drinking water dose, the ratio for AUC was 6.800 / 13.95 = 0.487 and for C_{max} was 0.3951 / 0.7316 = 0.540. These ratios were applied in Table 2-2 to calculate the POD_{HED} from the rat benchmark dose lower confidence limits (BMDLs) and no-observed-adverse-effect levels (NOAELs) for each endpoint.

Mouse to Human Extrapolations

The mouse and human PBPK models as described above were applied to derive HEDs for candidate PODs for endpoints selected from mouse bioassays. The mouse and human PBPK models were used to estimate two dose metrics—the area under the curve (AUC) and peak concentration (C_{max}) for RDX concentration in arterial blood. The relationships between administered dose and both internal metrics (AUC and C_{max}) were evaluated with the mouse PBPK model over the range 10 µg/kg-day to 100 mg/kg-day and with the human PBPK model over the range 0.05 µg/kg-day to 200 mg/kg-day, ranges that encompass the PODs. The times to reach steady state for the dose metrics were shorter than the duration of the toxicity studies, so the steady state values were considered representative of the study and were used. To calculate steady state values for daily exposure, the simulations were run until the daily average had a <1% change between consecutive days. For both the mouse and human PBPK models, both dose metrics correlated linearly with the administered dose. For mouse dosed via gavage, the slope of administered dose versus AUC was 0.0656 mg/L-day / mg/kg-day and that for C_{max} was 0.0273 mg/L / mg/kg-day. For a continuous dose, the slope of dose versus AUC was the same 0.0656 mg/L-day / mg/kg-day and for C_{max} was 0.0081 mg/L / mg/kg-day. For humans, assuming a drinking water dose sipping pattern, the slope of administered dose versus AUC was 13.95 mg/L-day / mg/kg-day and that for C_{max} was 0.7316 mg/L / mg/kg-day. Given this linearity in internal metrics and assuming that equal internal metrics in mice and humans are associated with the same degree of response, the HEDs could then be directly determined by multiplying the BMDL in mice by the ratio of these slopes. For a gavage dose in mice converted to a human drinking water dose, the ratio for AUC was 0.0656 / 13.95 =

0.0047 and C_{max} was 0.0273 / 0.7316 = 0.373, respectively. For a continuous dose in mice converted to a human drinking water dose, the ratio for AUC was 0.0656 / 13.95 = 0.0047 and for C_{max} was 0.0081 / 0.7316 = 0.011. These ratios were applied in Table 2-2 to calculate the POD_{HED} from the mouse BMDLs and NOAELs for each endpoint.

Summary of Confidence in PBPK Models for RDX

Overall, good fits to the rat, mouse, and human time-course data for RDX internal concentrations were obtained. For the rat and human models, calibration was based generally on fitting to more than one data set obtained from different studies originating in different laboratories or accidental exposure settings. Predictions from the rat model compared well with data from a subchronic drinking water study that was not used in model calibration.

The metabolic rate constant used in the human model was fit to limited data from accidentally exposed humans; however, the value of the metabolic rate constant has additional support from in vitro experimental data. The rat metabolic rate constant, fit to multiple experimental data sets, was scaled to humans using the ratio of human to rat rate constants measured with in vitro methods. This scaled value of the human metabolic rate constant was very similar to the rate constant estimated by fitting the model to the human data. The congruence in values increases the confidence in using the EPA-modified PBPK model for predicting human blood RDX concentrations.

There are several uncertainties in these models (listed below), the most significant of which pertain to the mouse PBPK model. The mouse model was based on a single data set, which used high RDX doses to obtain detectable RDX blood concentrations, and the types of additional data that increased the confidence in the rat and human models are not available for mice. The additional data not available for mice are in vitro measurements of RDX metabolism by mouse cells and quantification of potential routes of RDX elimination in mice. Overall, these uncertainties result in lower confidence in the mouse model than in the rat and human models.

1) RDX is readily metabolized in several species, yet there are no data on the toxicokinetics of RDX metabolites in the rat and human. Some data are available for the n-nitrosoamine metabolites (a minor metabolic pathway) in mice, but the data are too sparse to help better parameterize a PBPK model. Consequently, the PBPK models used in this assessment do not incorporate the kinetics of RDX metabolites.

2) The available toxicokinetic data are not sufficient to uniquely identify a parameter value for RDX oral bioavailability. Consequently, the model assumes 100% bioavailability even though some studies in rats suggest that a lower bioavailability is likely.

3) The human model is based on single accidental exposures, and the exposure concentrations are not known.

4) The only route of clearance of RDX used in the models is that of total metabolism, which appears reasonable for the rat for which only roughly 5% of the RDX was detected

unmetabolized in urine and feces. However, no data on the excretion of RDX are available for the mouse. This inability to properly characterize the fraction of RDX that is metabolized in the mouse is problematic considering some evidence to indicate that the role of metabolism in RDX toxicity may be different across species. This uncertainty decreases the confidence in the mouse PBPK model.

5) The PBPK model for the mouse is based on a single data set. This single data set is used to fit both the absorption and metabolic rate constants. There are no in vitro data to independently estimate the metabolic rate constant for the mouse. Consequently, the confidence in the mouse model parameter values is low.

6) The analytical detection limit in the mouse pharmacokinetic study is too high to enable detection at the lower doses. The lowest dose that resulted in a detectable level of RDX in blood was 35 mg/kg; this dose was high enough to manifest some toxicity in the chronic mouse bioassay. The measured blood concentration at the final 4-hour timepoint at the 35 mg/kg dose was based on the level measured from one animal only (in the other five animals exposed at this dose, three were non-detects, one was excluded as an outlier, and one animal died). Data from a single animal decreases the confidence in the calibration of the mouse PBPK model.

7) The metabolic rate constant as estimated by the PBPK model for mice was 30-fold higher than the rat (after accounting for body weight differences), suggesting that the toxicokinetics of RDX could be significantly different in the mouse than in the rat. Mice may have more efficient or higher expression of the CYP450 enzymes. Alternatively, mice may have other unknown metabolic pathways responsible for metabolizing RDX. Identifying the specific CYP450 enzymes and measuring expression levels and in vitro metabolic rate constants in mice would allow for in vitro scaling from rats to mice, which could be used to independently evaluate the mouse metabolic rate constant. Given the high sensitivity of the model to the metabolic rate constant, this uncertainty in the mouse toxicokinetics significantly decreases the confidence in using the mouse PBPK model for predicting mouse blood RDX concentrations.

Model Code for RDX PBPK Model Used in the Assessment

The PBPK acslX model code is made available electronically through the Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2014).

C.2. HUMAN STUDIES

Table C-8 presents a summary of case reports of humans acutely exposed to RDX. Table C-9 provides a chronological summary of the methodologic features of the available epidemiology studies of RDX.

Table C-8. Summary of case reports of exposure to RDX

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Barsotti and Crotti (1949) 17 males among 20 male Italian workers (1939–1942) Manufacturing	Inhalation of RDX powder during the drying, cooling, sieving, and packing processes of its manufacture	Generalized convulsions of a tonic-clonic (epileptic) type followed by postictal coma; loss of consciousness without convulsions; vertigo; vomiting and confusion; transient arterial hypertension Symptoms occurred either without prodromal symptoms or were preceded by several days of insomnia, restlessness, irritability, or anxiety	Tobacco and alcohol use were considered by the study authors to be aggravating factors
Kaplan et al. (1965) 5 males among 26 workers (April–July 1962) Manufacturing	Inhalation, ingestion, and possible skin absorption as a result of the release of RDX dust into the workroom air during the dumping of dried RDX powder, screening and blending, and cleanup of spilled material without adequate ventilation	Sudden convulsions or loss of consciousness without convulsions; few or no premonitory symptoms (e.g., headache, dizziness, nausea, vomiting); stupor, disorientation, nausea, vomiting, and weakness; no changes in complete blood counts or urinalysis	Mild cases of RDX intoxication may have been masked by viral illness with nonspecific symptoms (e.g., headache, weakness, upset GI tract); no method was available for determining RDX concentrations in air; recovery was complete without sequelae
Merrill (1968) 2 males Wartime, Vietnam	Ingestion of unknown quantity of C-4 with moderate amounts of alcohol	Coma, vomiting, hyperirritability, muscle twitching, convulsions, mental confusion, and amnesia; kidney damage (oliguria, gross hematuria, proteinuria, elevated BUN); liver or muscle damage (high AST); leukocytosis	Confounding factors included ingestion of C-4 while intoxicated with ethanol (vodka), which may have caused GI symptoms, and smoking (1–1.5 packs of cigarettes per day)

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Stone et al. (1969) 4 males (March–December 1968) Wartime, Vietnam	Ingestion of 180 g (patient 1), or 25 g (patients 2, 3) of C-4 (91% RDX)	Generalized seizures, lethargy, nausea, vomiting, fever, muscle soreness, headaches, twitching, (semi)comatose, headaches, hematuria, abnormal laboratory findings, muscle injury, elevated AST; no kidney damage For the patient who ingested the highest dose, anemia and loss of recent memory present after 30 d	Troops ingested small quantities of RDX to get a feeling of inebriation similar to that induced by ethanol
Hollander and Colbach (1969) 5 males (June 1968–January 1969) Wartime, Vietnam	Inhalation (all five cases) and ingestion of unknown quantity of C-4 (two cases)	Tonic-clonic seizures; nausea and vomiting occurred before and after admission; hyperirritability, muscle twitching, convulsions, mental confusion, and amnesia; kidney damage (oliguria, gross hematuria, proteinuria, elevated BUN); liver or muscle damage (high AST); leukocytosis; symptoms cleared by the next day except for amnesia (in case 2), oliguria (lasted for 4 d), and gross hematuria (decreased by 9th hospital day)	
Knepshield and Stone (1972) 6 males Wartime, Vietnam	Ingestion of C-4, range 25–180 g, average 77 g	Generalized seizures, coma, lethargy, severe neuromuscular irritability with twitching and hyperactive reflexes, myalgia, headache, nausea, vomiting, oliguria, gross hematuria, low-grade fever; abnormal laboratory findings (neutrophilic leukocytosis, azotemia, elevated AST)	Includes data on two patients from Merrill (1968)
Ketel and Hughes (1972) 18 males (December 1968–December 1969) Wartime, Vietnam	Inhalation while cooking with C-4 and possible ingestion	CNS signs (confusion, marked hyperirritability, involuntary twitching of the extremities, severe prolonged generalized seizures, prolonged postictal mental confusion, amnesia); renal effects (oliguria and proteinuria, one case of acute renal failure requiring hemodialysis); GI toxicity (nausea, vomiting)	C-4 was cut with the same knife used to stir/prepare food

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Woody et al. (1986) 1 male child (August 1984) Manufacturing	Ingestion of plasticized RDX from mother's clothing and/or boots; estimated ingested dose of 1.23 g RDX was normalized to the patient's body weight (84.82 mg/kg)	Seizures before and after admission; EEG revealed prominent diffuse slowing that was greatest in the occipital regions with no evidence of epileptiform activity; elevated AST on admission and after 24 hrs; within 24 hrs, the child was extubated and intensive care withdrawn; normal mental status and normal neurological examination at discharge	Mother worked at an explosive plant in which RDX was manufactured in a plasticized form
Goldberg et al. (1992) 1 male Nonwartime	Ingestion after chewing a piece (unknown size) of "Semtex" plastic explosive 4 hrs before first seizure	Frontal headache and two tonic-clonic seizures; progressively disseminating petechial rash suggestive of meningococcal infection apyrexial; normotensive; no photophobia; no neurological abnormalities; florid petechial rash over the face and trunk; lacerated tongue Initial results included leukocyte count of 10.8×10^9/dL (87% neutrophils); hemoglobin, platelet count, coagulation screen, serum and CSF biochemistry all within normal limits; CSF and blood bacteriologically unremarkable Shortly following admission, headache and rash disappeared; no further seizures	
Harrell-Bruder and Hutchins (1995) 1 male Nonwartime	Ingestion of C-4 (chewing on a piece of undetermined size)	Tonic-clonic seizures; postictal state; EEGs were normal; brisk deep tendon reflexes	
Testud et al. (1996a) 1 male Manufacturing	Inhalation and possible dermal exposure during the RDX manufacturing process	Malaise with dizziness, headache, and nausea progressing to unconsciousness and generalized seizures without involuntary urination or biting of the tongue; blood chemistries were in the normal range and blood alcohol content was null	

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Testud et al. (1996b) 2 males Manufacturing	Inhalation and possible dermal exposure during the RDX manufacturing process	Sudden loss of consciousness and generalized seizures; blood serum level of 2 mg/L RDX measured	Smoker and alcohol drinker
Hett and Fichtner (2002) 1 male Nonwartime	Ingestion of a cube (1 cm across) of C-4	Nausea and vomiting; tonic-clonic seizure lasting 2 min, followed by two seizures of about 30 sec each; myoclonic jerks in all limbs; petechial hemorrhages around face and trunk after seizures	
Küçükardali et al. (2003) 5 males Nonwartime	Ingestion (accidental) of 37−250 mg/kg body weight RDX during military training via food contaminated with RDX	Abdominal pain, nausea, vomiting, myalgia, headache, generalized weakness, repetitive tonic-clonic convulsions, lethargic or comatose between seizures, hyperactive deep tendon reflexes, sinusal tachycardia; elevated serum levels of AST and ALT; kidney damage; plasma RDX levels 3 hrs after ingestion ranged from 268 to 969 pg/mL	
Davies et al. (2007) 17 males Nonwartime	Ingestion of unknown quantity C-4 under unclear circumstances, but unrelated to recreational abuse	Seizures, headache, nausea, and vomiting; hypokalemia and elevated creatine kinase, lactate dehydrogenase, and phosphate noted in all but two patients; metabolic acidosis only occurred in two patients directly following seizures	Patient histories may have been affected by the fact that the incident was the focus of a military police investigation
Kasuske et al. (2009) 2 males Nonwartime	Ingestion of C-4 after handling explosive ordnance	Seizures, postictal state, confusion, drowsiness, headache, nausea, and vomiting; blood work revealed high WBC count and elevated creatine phosphokinase; proteinuria and gross hematuria observed	

ALT = alanine aminotransferase; AST = aspartate transaminase; BUN = blood urea nitrogen; CNS = central nervous system; CSF = cerebrospinal fluid; EEG = electroencephalogram; WBC = white blood cell

1 **Table C-9. Occupational epidemiologic studies of RDX: summary of methodologic features**

Reference, setting and design	Participants, selection, follow-up	Consideration of likely selection bias	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results details	Sample size
Ma and Li (1993) (China)[a] Industrial workers (translated article)	Details of industrial process and subject selection framework not reported; referents chosen from same plant; age, employment duration, and education similar across groups; participation rate not reported	Sparse reporting of details on subject recruitment and participation	Details of exposure monitoring not reported. Two groups of exposed subjects: Group A, intensity, 0.407 (0.332) mg/m^3 [mean (standard deviation)], daily cumulative, 2.66 (1.89) mg/m^3. Group B, intensity, 0.672 (0.556) mg/m^3; daily cumulative, 2.53 (8.40) mg/m^3.	Neurobehavioral battery administered by trained personnel: memory retention, simple reaction time, choice reaction time, letter cancellation, and block design	No adjustment for other risk factors (e.g., alcohol consumption); no consideration or attempt to distinguish TNT	Comparisons of mean scaled score on memory retention, letter cancellation, or block design test; mean time on reaction tests; and total behavioral score; variance (F test), linear and multiple regression, and correlation analysis	60 exposed; Group A (n = 30; 26 males, 4 females); Group B (n = 30); 32 referents (27 males, 5 females)
Hathaway and Buck (1977) (United States) Ammunition workers	2,022 workers (1,017 exposed to open explosives (TNT, RDX, HMX), 1,005 referents) at five U.S. Army ammunition plants (Iowa, Illinois, Tennessee); participation rate: 76% exposed, 71% referents	Potential healthy worker effect	Atmospheric samples of all operations with potential exposure to open explosives taken in 1975. Range: not detected to 1.57 mg/m^3. Seventy exposed workers with RDX at >0.01 mg/m^3 [the LOD]; mean: 0.28 mg/m^3 [standard deviation not presented]. Job title used to initially identify exposed or unexposed status and reassigned to one of two	Liver function, renal function, and hematology tests [blood]	Workers with TNT exposure excluded from exposed groups	Comparison of mean value; prevalence of elevated value on an individual test	69 RDX exposed (43 males, 26 females), 24 RDX/HMX exposed (all males), 338 referents (237 males, 101 females)

Reference, setting and design	Participants, selection, follow-up	Consideration of likely selection bias	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results details	Sample size
			exposed groups (nondetected, >0.01 mg/m^3) based on subject's industrial hygiene monitoring results.				
West and Stafford (1997) (United Kingdom) Ammunition workers (case-control study)	378 of 404 subjects (excluded 3 deaths and 23 subjects with unknown addresses) previously studied in 1991, 32 cases with abnormal hematology test and 322 controls with normal hematology test; participation rate among eligible subjects: 97% cases, 93% controls	Former employees who were unable to work due to adverse health outcome were not included in the 1991 prevalence study	Semiquantitative assessment; source of industrial hygiene data not reported. Interviews with current and past employees and job title analysis were conducted to identify potential exposure to 100 agents, including RDX. Exposure surrogate was >50 hrs in duration and intensity was low (1−10 ppm), moderate (10−100 ppm), or high (100−1,000 ppm). RDX exposure prevalence (males) was 83%.	Abnormal hematology value in 1991 survey indicating possible myelodysplasia: neutropenia (2.0 x 10^9/L), low platelet count (<150 x 109/L), or macrocytosis (mean corpuscular volume = 99 fL or >6% macrocytes)	Cases and controls were not matched and statistical analyses were not adjusted for other risk factor or occupational exposures; no consideration or attempt to distinguish TNT	Unadjusted prevalence odds ratios and 95% CIs; analyses limited to males because of low frequency of exposure to females	32 cases (29 males, 3 females) and 322 controls (282 males, 12 females)

[a]Ma and Li (1993) describe symptoms reported by subjects during a physical examination, but these are not included in the evidence table because responses for individual symptoms were not identified.

CI = confidence interval; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; LOD = limit of detection; TNT = trinitrotoluene

C.3. OTHER PERTINENT TOXICITY INFORMATION

C.3.1. Mortality in Animals

Evaluations of the evidence for specific health effects associated with RDX exposure are provided in Sections 1.2.1 to 1.2.6. In addition to these specific organ/system health effects, reduced survival associated with RDX exposure has been observed in experimental animals across multiple studies of varying exposure duration and study design (Table C-10). Evidence pertaining to mortality in experimental animals exposed to RDX is summarized in Table C-10; studies are ordered in the evidence table by duration of exposure and then species.

Following chronic dietary exposure, an increased rate of mortality in F344 rats, and in particular male rats, at 40 mg/kg-day was largely attributed to RDX-related effects on the kidneys (Levine et al., 1983)[4]; see further discussion in Section 1.2.2. In a comparable chronic study, mice were less sensitive than rats with respect to mortality following RDX exposure. After the high dose was reduced from 175 to 100 mg/kg-day at week 11 in a 2-year dietary study in B6C3F$_1$ mice because of high mortality, the mortality curve at 100 mg/kg-day in surviving mice was not significantly different from the control group for the duration of the 2-year study (Lish et al., 1984). The investigators did not identify the probable cause of death at 175 mg/kg-day.

Increased rates of mortality were also observed in experimental animals that ingested RDX for durations up to 6 months (Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Cholakis et al., 1980; von Oettingen et al., 1949). The most detailed data on RDX-related mortality come from a 90-day gavage study in F344 rats by Crouse et al. (2006). Across groups of rats exposed to 8–15 mg/kg-day RDX, pre-term deaths occurred in male rats as early as day 26–78 and in female rats as early as day 8–16 (Johnson, 2015; Crouse et al., 2006). Treatment-related mortality was also observed in the dams of rats exposed gestationally by gavage at doses ranging from 20 to 120 mg/kg-day (Angerhofer et al., 1986; Cholakis et al., 1980). Deaths were additionally reported in one of 40 pregnant dams in both 2 and 6 mg/kg-day groups in the rat developmental toxicity study by Angerhofer et al. (1986)

In general, the evidence suggests that mortality occurs at lower doses in rats than in mice (e.g., comparison of rates from the 2-year dietary studies in mice by Lish et al. (1984) and in rats by Levine et al. (1983)), and at lower doses following gavage administration than dietary administration (e.g., comparison of rates from the 13-week rat studies using gavage (Crouse et al., 2006) and dietary (Levine et al., 1981a) administration). An RDX formulation with a larger particle size (e.g., ~200 µm) (Cholakis et al., 1980), which could potentially reduce the ability of RDX to

[4] Deaths in high-dose (40 mg/kg-day) male rats were reported as early as week 4 (estimated from Volume 1, Figure 4 in Levine et al. (1983)); the cause of death in rats that died prior to 6 months on study was generally not determined (Levine et al., 1983). Survival rates in both male and female rats at doses ≤8 mg/kg-day RDX were similar to the control.

enter the bloodstream, appears to produce less mortality than formulations with finer particle sizes (e.g., median particle diameter of 20 µm) (Levine et al., 1981a). There is evidence that mortality may be associated with nervous system effects; several investigators reported that unscheduled deaths were frequently preceded by convulsions or seizures (Crouse et al., 2006; Levine et al., 1983; Cholakis et al., 1980). In a number of studies, treatment-related mortality was observed at doses as low as doses associated with 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). The evidence for an association between nervous system effects and mortality is discussed in more detail in Section 1.2.1, Nervous System Effects.

In humans, there were no reports of mortality in case reports involving workers exposed to RDX during manufacture or in individuals exposed acutely as a result of accidental or intentional ingestion (see Appendix C, Section C.2).

Table C-10. Evidence pertaining to mortality in animals[a]

Reference and study design	Results					
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 (mortality incidence also provided for mice through week 11 when the high dose was dropped because of high mortality at that dose, and from the report of the 6-month interim sacrifice)	Doses	0	1.5	7.0	35	175/100
	Mortality at 11 wks *(incidence)*					
	M	1/85	0/85	0/85	0/85	30/85
	F	0/85	0/85	0/85	0/85	36/85
	Mortality at 6 mo *(incidence)*					
	M	1/85	2/85	3/85	3/85	34/85
	F	0/85	1/85	0/85	0/85	36/85
	Mortality at 2 yrs *(incidence)*					
	M	20/65	23/65	25/65	29/65	41/65
	F	16/65	21/65	14/65	21/65	42/65
	After the high dose was reduced to 100 mg/kg-d, survival was similar to controls.					
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 (mortality incidence also provided for mice through week 13, and from the report of the 6-month scheduled sacrifice)	Doses	0	0.3	1.5	8.0	40
	Mortality at 13 wks *(incidence)*					
	M	0/75	0/75	0/75	0/75	0/75
	F	0/75	0/75	0/75	0/75	0/75
	Mortality in 6-mo interim sacrifice animals *(incidence)*[#] [#]includes spontaneous death and moribund sacrifice					
	M	0/75	0/75	0/75	0/75	5/75
	F	0/75	0/75	0/75	0/75	0/75
	Mortality at 2 yrs *(incidence)*					

Reference and study design	Results					
	M	17/55	19/55	30/55*	26/55	51/55*
	F	12/55	10/55	13/55	14/55	27/55*
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)[b] Diet 13 wks	Doses	0	79.6		147.8	256.7
	Mortality *(incidence)*					
	M	0/10	0/10		0/10	4/10*
	F	0/11	0/12		0/10	2/12
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
	Mortality *(incidence)*						
	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	1/10 (accidental death)	0/10	0/10	0/10	0/10	0/10
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
	Mortality in F0 adults *(incidence)*[c]						
	M (F0)	0/22	0/22		0/22		2/22
	F (F0)	0/22	0/22		0/22		6/22
	M + F (F0)	0/44	0/44		0/44		8/44*
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
	Mortality *(incidence)*						
	M	0/10	0/10	1/10	3/10	2/10	3/10
	F	0/10	0/10	1/10	2/10	5/10	4/10
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[d] Rats, F344, 10/sex/group; 30/sex for control	Doses	0	10	30	100	300	600
	Mortality *(incidence)*[e]						
	M	0/30	0/10	0/10	8/10	10/10	10/10

Reference and study design	Results						
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	F	0/30	1/10	0/10	5/10	10/10	10/10
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	Doses	0		15	25		50
	Mortality *(incidence)*						
		0/20		1/20 (probably not related to RDX)	8/20		8/20
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	Doses	0		0.1	1		10
	Mortality *(incidence)*						
	M	0/3		0/3	1/3 (not related to RDX)		0/3
	F	0/3		0/3	0/3		0/3
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus[f], 3/sex/group Purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0		0.1	1		10
	Mortality *(incidence)*						
	M	0/3		0/3	0/3		0/3
	F	0/3		0/3	0/3		1/3 (animal exhibited neurological effects; euthanized)
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 not specified 0 or 50 mg/kg-d Diet 6 d/wk for 6 wks	Doses	0					50
	Mortality *(incidence)*						
	F	0/5					1/7
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 mortality was reported (incidence data were not provided).						

Reference and study design	Results					
Cholakis et al. (1980) Rabbits, New Zealand White (NZW), 11–12 pregnant females/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 Diet GDs 7–29	Doses	0	0.2	2.0	20	
	Mortality *(incidence)*					
	F	0/11	0/11	0/11	0/12	
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	Doses	0	0.2	2.0	20	
	Mortality (incidence)					
	F	0/24	0/24	0/24	5/24 (1 rat accidentally killed; removed from analysis)	
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	Doses	0	10	20	40	80	120
	Mortality (incidence)						
	F	0/6	0/6	0/6	6/6	6/6	6/6
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	Doses	0	2	6	20	
	Mortality (incidence)					
	F	0/39	1/40	1/40	16/51	
		Not reported whether deaths at 2 and 6 mg/kg-d related or likely related to RDX exposure				

*Statistically significant ($p < 0.05$) based on analysis by the study authors.
[a]The 2-year rat study by Hart (1976) was not included in this evidence table because a malfunctioning heating system incident resulted in the premature deaths of 59 animals on study days 75–76 across groups, thereby confounding mortality findings.
[b]Doses were calculated by the study authors.
[c]Data for male and female rats were combined for statistical analysis.
[d]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.
[e]Animals receiving 300 mg/kg-day died by week 3 of the study; animals receiving 600 mg/kg-day died by week 1 of the study.
[f]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).

TWA = time-weighted average

C.3.2. Other Noncancer Effects

There are isolated reports of RDX inducing systemic effects in several organs/systems, including the eyes and the musculoskeletal, cardiovascular, immune, and GI systems. However, there is less evidence for these effects compared to organ systems described in Section 1.2. Overall, at the present time, the evidence does not support identifying these other systemic effects as human hazards of RDX exposure. Summaries of the evidence for other systemic effects in humans and animals are shown in Tables C-11 and C-12, respectively. Experimental animal studies are ordered in the evidence table by type of effect, and then by duration of exposure and by species.

Ocular Effects

There are no reports of ocular effects in human case reports or epidemiological studies. In experimental animals, evidence of ocular effects comes from cataract findings in one 2-year bioassay. Specifically, the incidence of cataracts was 73% in female F344 rats exposed to 40 mg/kg-day RDX in the diet for 2 years, compared with 32% in the control group (Levine et al., 1983). After 76 weeks of exposure, the incidence of cataracts in female rats at 40 mg/kg-day (23%) was also elevated compared to controls (6%). The incidence of cataracts was not increased in RDX-exposed male rats in the same study (Levine et al., 1983), and other studies have not observed ocular effects associated with RDX exposure. Only two rats (dose groups not reported) were observed to have mild cataracts in a 90-day study of male and female F344 rats exposed to RDX at doses up to 15 mg/kg-day by gavage; however, the authors noted that these observations are common in F344 rats at 4 months of age and should not be attributed to treatment (Crouse et al., 2006). Furthermore, cataracts were not observed in male or female F344 rats exposed to 40 mg/kg-day RDX by diet for 90 days (Cholakis et al., 1980) or in male or female $B6C3F_1$ mice exposed to RDX in the diet for 2 years at doses up to approximately 100 mg/kg-day (Lish et al., 1984). A statistically significant increase in the incidence of cataracts in male mice was initially noted by Lish et al. (1984), but was not confirmed when mice used for orbital bleedings were excluded from the analysis, suggesting that the effect was not treatment related. No ocular effects were observed in monkeys exposed by gavage for 90 days at doses up to 10 mg/kg-day (Martin and Hart, 1974).

In summary, the incidence of cataracts was statistically significantly increased in high-dose female rats in one chronic oral study; however, this finding was not reproduced in other subchronic and chronic studies in rats or mice.

Cardiovascular Effects

Human evidence for cardiovascular effects is limited to case reports that include observations of transient arterial hypertension in male workers following inhalation of RDX during manufacturing (Barsotti and Crotti, 1949), sinus tachycardia, and in one instance, premature ventricular beats in five men following accidental ingestion of RDX at 37–250 mg/kg body weight (Küçükardali et al., 2003) (see Appendix C, Section C.2).

Inconsistent observations of cardiovascular effects have been reported in animal studies. An increase in the relative heart-to-body weight ratio was observed at the highest dose tested in $B6C3F_1$ mice (male: 13%; female: 17%) and F344 rats (male: 22%; female: 15%) following chronic dietary administration of RDX (Lish et al., 1984; Levine et al., 1983); however, these doses also resulted in reductions in body weight in both males and females. Dose-related decreases in absolute heart weight in rats were reported in some subchronic (dietary) studies (Levine et al., 1990; Levine et al., 1981a, b; Cholakis et al., 1980), whereas little change or modest increases in absolute heart weight were observed in other subchronic studies in rats or mice (Crouse et al., 2006; Cholakis et al., 1980). A subchronic study in male dogs reported a 31% increase in absolute heart weight at the highest dose tested (10 mg/kg-day) (Hart, 1974).

Evidence for changes in histopathology associated with RDX exposure is limited to findings of an increased incidence of focal myocardial degeneration in female rats (6/10 versus 2/10, respectively) and male mice (5/10 versus 0/10, respectively) compared with controls following exposure to RDX in the diet for 90 days (Cholakis et al., 1980). With the exception of one male mouse, the severity of the lesion was characterized as minimal. In each study, the finding of myocardial degeneration was limited to one sex and to the high-dose group only; the high dose in the male mouse study caused 40% mortality. Other studies in monkeys (Martin and Hart, 1974) and rats (von Oettingen et al., 1949) reported no observable cardiovascular effects.

In summary, evidence for cardiovascular effects associated with RDX exposure consists of two case reports of cardiovascular effects following acute exposure, inconsistent findings of changes in heart weight in experimental animals, and one report of minimal histopathological changes in a 90-day rat study that was not confirmed in other toxicity studies.

Musculoskeletal Effects

Evidence of musculoskeletal effects in humans consists of case reports that include observations of muscle twitching, myalgia/muscle soreness, and muscle injury as indicated by elevated levels of aspartate aminotransferase (AST) or myoglobinuria (Küçükardali et al., 2003; Hett and Fichtner, 2002; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968) (see Appendix C, Section C.2). Histological evaluations of musculature or skeletal tissue did not reveal any alterations in mice (Lish et al., 1984) or rats (Levine et al., 1983; Hart, 1976) following chronic oral exposure to RDX, in mice and rats following subchronic exposure (Cholakis et al., 1980), or in dogs following a 90-day dietary exposure (Hart, 1974).

Immune System Effects

RDX is structurally similar to various drugs known to induce the autoimmune disorder systemic lupus erythematosus (SLE). Based on the initial identification of three cases of SLE at one U.S. Army munitions plant, further investigation was conducted on a population of 69 employees at five U.S. Army munitions plants with potential exposure to RDX (Hathaway and Buck, 1977); no additional cases of SLE were identified. Increased WBC counts have been reported

in some case reports of individuals (troops during the Vietnam war) who ingested or inhaled RDX or C-4 (91% RDX) (Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968).

In animal studies, increased WBC count in female rats following subchronic dietary exposure to RDX was the only dose-related immune effect reported (Levine et al., 1990; Levine et al., 1981a, b); WBC counts in male rats were unaffected. Conversely, decreased WBC counts were reported in male and female rats in a 2-year study (Hart, 1976). Changes in spleen weights were observed across studies, but the responses were not consistent and did not appear to be dose-related. For example, in 90-day studies, Cholakis et al. (1980) identified a statistically significant decrease in absolute spleen weight in female F344 rats at 40 mg/kg-day, while Crouse et al. (2006) observed a statistically significant increase in spleen weight at >10 mg/kg-day. Across studies, there was no significant or dose-dependent pattern of response to suggest that the WBC changes reflect RDX-induced immunotoxicity. No dose-related immune effects from oral exposure to RDX were observed in other animal studies, including a 90-day study in F344 rats specifically designed to evaluate immunotoxicity (parameters included evaluation of 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). Routine clinical and histopathology evaluations of immune-related organs in a two-generation study in rats (Cholakis et al., 1980) and chronic studies in rats (Levine et al., 1983) and mice (Lish et al., 1984) provide no evidence of immunotoxicity associated with oral (dietary) exposure to RDX.

In summary, evidence for immunotoxicity associated with RDX exposure is limited to findings from one study of increased WBC counts in female rats (Levine et al., 1981a, b). Evidence that RDX is not immunotoxic comes from several animal studies, including other repeat-dose oral studies in mice and rats (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Cholakis et al., 1980).

Gastrointestinal Effects

Clinical signs of nausea and/or vomiting have been frequently identified in case reports of accidental or intentional RDX poisonings, and have generally been 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) (see Appendix C, Section C.2). In animal studies, vomiting has also been observed following oral exposure in swine (single-dose study) (Musick et al., 2010), dogs (Hart, 1974), and monkeys (Martin and Hart, 1974). One subchronic oral (diet) rat study from the early literature reported congestion of the GI tract at doses also associated with elevated mortality (von Oettingen et al., 1949); however, none of the subsequent subchronic or chronic animal studies reported histological changes of the GI tract

related to RDX administered via gavage or the diet (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Hart, 1974; Martin and Hart, 1974).

In summary, evidence for GI tract effects associated with RDX exposure consists largely of reports of nausea and vomiting in humans acutely exposed to RDX and similar reports of vomiting in swine, dogs, and monkeys. In general, histopathological changes have not be reported in experimental animals exposed to RDX in the diet.

Hematological Effects

Elevated prevalence odds ratios (ORs) for hematological abnormalities (i.e., neutropenia, low platelet count, or macrocytosis; see Table C-11 for criteria used to define abnormal) were observed in a case-control study of men (24 cases, 199 controls) exposed to RDX in ordnance factories (West and Stafford, 1997) (see Table C-11). The prevalence OR for an association between RDX exposure and hematological abnormalities was 1.7 (95% confidence interval [CI] 0.7–4.2) for men with >50 hours of low-intensity exposure (based on 22 cases), while the prevalence OR was 1.2 (95% CI 0.3–5.3) for men with >50 hours of high-intensity exposure (based on 2 cases). The ORs from this study must be interpreted with caution given the small sample size, wide CIs, and lack of identification of co-exposures. No changes in hematological parameters (including hemoglobin, hematocrit, and reticulocyte count) were observed in a cross-sectional epidemiologic study of 69 workers exposed to RDX by inhalation (RDX exposure range: undetectable [<0.01 mg/m^3] to 1.6 mg/m^3) (Hathaway and Buck, 1977). Humans who ingested or inhaled unknown amounts of RDX or C-4 (~91% RDX) for an acute duration displayed temporary hematological alterations, including anemia, decreased hematocrit, hematuria, and methemoglobinemia (Kasuske et al., 2009; Küçükardali et al., 2003; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968). In other case reports, normal blood counts were observed in accidentally exposed individuals (Testud et al., 1996a; Goldberg et al., 1992; Woody et al., 1986; Ketel and Hughes, 1972; Kaplan et al., 1965) (see Appendix C, Section C.2).

In animals, hematological alterations were observed following oral exposure in chronic and subchronic studies in both sexes of rats (F344 or Sprague-Dawley) and B6C3F$_1$ mice (see Table C-12). Increases in platelet count were observed in male and female mice and rats in some subchronic and chronic studies at doses ranging from 0.3 to 320 mg/kg-day (Lish et al., 1984; Levine et al., 1983; Cholakis et al., 1980); however, changes were generally inconsistent across studies and were not generally dose-dependent. Similarly, decreased hemoglobin levels/anemia were observed in some chronic and subchronic studies (Levine et al., 1983; Cholakis et al., 1980; von Oettingen et al., 1949), particularly at doses ≥15 mg/kg-day, but trends in hemoglobin levels across studies did not show a consistent relationship with dose. Other hematological parameters, including WBC counts, reticulocyte counts, and hematocrit, showed conflicting results between studies, marginal responses, or inconsistent changes with increasing dose. Other subchronic

studies in rats and dogs (Crouse et al., 2006; Hart, 1974; von Oettingen et al., 1949) did not identify any changes in hematological parameters.

In summary, evidence for hematological effects associated with RDX exposure in humans comes from several case reports that found transient fluctuations in hematological endpoints after acute exposures. Hematological findings from the case-control study and the cross-sectional study were not consistent. The small number of cases or exposed individuals, respectively, from the case-control and cross-sectional study may contribute to the difficulty in interpreting the results across studies (Table C-11). In general, animal studies of chronic and subchronic durations showed no consistent, dose-related pattern of increase or decrease in hematological parameters.

Table C-11. Evidence pertaining to other noncancer effects (hematological) in humans

Reference and study design	Results	
Hematological effects		
West and Stafford (1997) (United Kingdom) Case-control study of ordnance factory workers, 32 cases with abnormal and 322 controls with normal hematology test drawn from 1991 study of 404 workers at ammunitions plant; participation rate 97% of cases, 93% of controls. Analysis limited to men (29 cases, 282 controls). Analysis specific to RDX: 22 low- and 2 high-intensity cases; 182 low- and 17 high-intensity controls. **Exposure measure**s: Exposure determination based on employee interviews and job title analysis; data included frequency (hrs/d, d/yr), duration (yrs), and intensity (low [1–10 ppm], moderate [10–100 ppm], and high [100–1,000 ppm], based on ventilation considerations). **Effect measures**: Hematology tests; blood disorder defined as neutropenia (2.0×10^9/L), low platelet count ($<150 \times 10^9$/L), or macrocytosis (mean corpuscular volume = 99 fl or >6% macrocytes). **Analysis**: Unadjusted OR.	**Hematological abnormality** (neutropenia, low platelet count, or macrocytosis) *(OR; 95% CI [number of exposed cases])*	
	Low intensity, 50-hr-duration	1.7; 0.7,4.2 [22]
	Medium intensity, 50-hr duration	1.6; not reported [not reported]
	High intensity, 50-hr duration	1.2; 0.3, 5.3 [2]

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td rowspan="6">Hathaway and Buck (1977) (United States) Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate). Analysis 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: Hematology 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>**Hematology tests 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>Hemoglobin</td><td>15.2</td><td>14.7</td><td>15.2</td></tr><tr><td>Hematocrit</td><td>42</td><td>45.6</td><td>47</td></tr><tr><td>Reticulocyte count</td><td>0.7</td><td>0.9</td><td>0.7</td></tr></table></td></tr>
<tr><td>*Includes both workers exposed to RDX alone and RDX and HMX.
No differences were statistically significant. Similar results in women.</td></tr>
<tr><td>**Hematology tests in men** *(prevalence of abnormal 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>Hemoglobin (<14)</td><td>15/237</td><td>3/22</td><td>4/45</td></tr><tr><td>Hematocrit (<40)</td><td>1/237</td><td>1/22</td><td>1/45</td></tr><tr><td>Reticulocyte count (>1.5)</td><td>18/237</td><td>3/22</td><td>2/45</td></tr></table></td></tr>
<tr><td>*Includes both workers exposed to RDX alone and RDX and HMX.
No differences were statistically significant. Similar results in women.</td></tr>
</table>

Table C-12. Evidence pertaining to other noncancer effects in animals[a]

Reference and study design	Results					
Ocular 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	Doses	0	1.5	7.0	35	175/100
	Cataracts; 103 wks *(incidence)*[b]					
	M	2/47	2/41	0/41	2/37	2/16
	F	2/50	1/37	6/52	0/46	1/26
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
	Cataracts; 103 wks *(incidence)*					
	M	8/40	6/39	6/31	8/35	2/6
	F	14/44	4/48	11/44	8/43	22/30*
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 ocular effects were observed (gross examination of eye was performed in all animals, and microscopic examination was performed in control and 40 mg/kg-d animals).					
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	No ocular effects were observed (ophthalmic examinations were performed in all animals within 1 wk of sacrifice, and microscopic examination of the eye was performed in control and 15 mg/kg-d animals).					
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	No ocular effects were observed (ophthalmoscopic examination was performed at the end of exposure).					

Reference and study design	Results					
Cardiovascular 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	Doses	0	1.5	7.0	35	175/100
	Absolute heart weight; 104 wks *(percent change compared to control)*					
	M	0%	4%	4%	5%	7%
	F	0%	1%	5%	2%	−5%
	Relative heart-to-body weight; 104 wks *(percent change compared to control)*					
	M	0%	7%	5%	5%	13%*
	F	0%	0%	6%	4%	17%*
	Body weight was significantly lower at termination in males and females exposed to 175/100 mg/kg-d (−5 and −19%, respectively).					
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	
	Myocardial fibrosis *(percent incidence; number not reported)*					
	M	20%	–	–	5%	
	F	5%	–	–	1%	
	Endocardial disease *(percent incidence; number not reported)*					
	M	1%	–	–	3%	
	F	0%	–	–	0%	
	Absolute heart weight; 104 wks *(percent change compared to control)*					
	M	0%	−6%	−2%	−5%	
	F	0%	13%	3%	15%	
	Relative heart-to-body weight; 104 wks *(percent change compared to control)*					
	M	0%	−2%	4%	1%	
	F	0%	23%	13%	27%	
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 heart weight; 104 wks *(percent change compared to control)*					
	M	0%	3%	−2%	−2%	1%
	F	0%	−1%	0%	−4%	−3%
	Relative heart-to-body weight; 104 wks *(percent change compared to control)*					
	M	0%	2%	6%	0%	22%
	F	0%	−2%	3%	−1%	15%

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 heart weight *(percent change compared to control)*						
	M	0%	–	–	–	7%	7%
	F	0%	–	–	–	0%	0%
	Relative heart weight *(percent change compared to control)*						
	M	0%	–	–	–	6%	0%
	F	0%	–	–	–	−4%	0%
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)[d] Diet 13 wks	Doses	0		80	160		320
	Focal myocardial degeneration *(incidence)*						
	M[#]	0/10		–	–		5/10*,‡
	F[†]	0/11		–	–		2/11
	Absolute heart weight *(percent change compared to control)*						
	M	0%		0%	0%		8%
	F	0%		0%	0%		8%
	Relative heart-to-body weight *(percent change compared to control)*						
	M	0%		0%	−2%		6%
	F	0%		0%	−2%		2%
	[#]Includes one affected and three unaffected animals that died prematurely. [†]Includes one unaffected animal that died prematurely. [‡]Minimal severity in four rats, moderate in one.						
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
	Focal myocardial degeneration, minimal severity *(incidence)*						
	M	3/10	–	–	–	–	1/10
	F	2/10	–	–	–	–	6/10
	Absolute heart weight *(percent change compared to control)*						
	M	0%	–	–	–	0%	−8%*
	F	0%	–	–	–	−6%	−11%*
	Relative heart-to-body weight *(percent change compared to control)*						
	M	0%	–	–	–	3%	0%
	F	0%	–	–	–	−3%	−8%
	Relative heart-to-brain weight *(percent change compared to control)*						
	M	0%	–	–	–	−4%	−10%*
	F	0%	–	–	–	−5%	−11%*

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 cardiac effects were observed (microscopic examination of heart was performed in randomly selected F2 animals).						
	Doses	0		5		16	50
	Absolute heart weight *(percent change compared to control)*						
	F2 M	0%		3.2%		−6.5%	–
	F2 F	0%		15%		−3.7%	–
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
	Cardiomyopathy *(incidence)*						
	M	2/10	–	–	–	–	3/8
	F	0/10	–	–	–	–	1/6
	Absolute heart weight *(percent change compared to control)*						
	M	0%	−2%	−7%	−1%	1%	11%
	F	0%	−2%	0%	8%	7%	6%
	Relative heart-to-body weight *(percent change compared to control)*						
	M	0%	4%	2%	1%	−1%	8%
	F	0%	−2%	−7%	−6%	−9%	−16%*
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[e] 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	All animals in the 300 and 600 mg/kg-d groups died prior to study termination.						
	Doses	0	10	30	100	300	600
	Chronic focal myocarditis *(incidence)*						
	M	8/30	8/10	6/10	1/10	1/10	0/10
	F	8/30	3/10	1/10	1/10	1/10	1/9
	Absolute heart weight *(percent change compared to control)*						
	M	0%	−2%	−10%	−15%	–	–
	F	0%	−3%	0%	−5%	–	–
	Relative heart-to-body weight *(percent change compared to control)*						
	M	0%	2%	−4%	3%	–	–
	F	0%	−2%	0%	−3%	–	–

Reference and study design	Results				
von Oettingen et al. (1949) Rats (sex/strain not specified); 20/group Purity and particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	The study authors reported that there were no cardiac effects (microscopic examination of the heart was performed in all rats; data were not shown).				
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	Doses	0	0.1	1	10
	Focal hyalinization of the heart *(incidence)*				
	M	0/3	–	–	0/3
	F	0/3	–	–	1/3
	Absolute heart weight *(percent change compared to control)*				
	M	0%	–	–	31%
	F	0%	–	–	5.7%
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
	Myocarditis *(percent change compared to control)*				
	M	1/3	–	–	1/3
	F	0/3	–	–	0/3
	Absolute heart weight *(percent change compared to control)*				
	M	0%	7%	−1%	5%
	F	0%	10%	12%	−12%
Immune 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	No immune effects were observed with routine hematology, clinical chemistry, or histopathology evaluations.				
	Doses	0	1.5	7.0	35	175/100
	WBC count; 105 wks *(percent change compared to control)*					
	M	0%	−13%	−8%	−16%	−30%
	F	0%	12%	39%*	28%	0%
	Absolute spleen weight; 105 wks *(percent change compared to control)*					
	M	0%	24%	31%	−10%	−28%
	F	0%	4%	15%	−17%	16%
	Relative spleen weight; 105 wks *(percent change compared to control)*					
	M	0%	26%	32%	−11%	−21%
	F	0%	4%	15%	−17%	44%

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	**Doses**	**0**	**1.0**	**3.1**	**10**	
	WBC count; 104 wks *(percent change compared to control)*					
	M	0%	−13%	−22%*	−34%*	
	F	0%	5%	−32%*	−12%	
	Absolute spleen weight; 104 wks *(percent change compared to control)*					
	M	0%	−11%	−16%	−4%	
	F	0%	58%	8%	37%	
	Relative spleen weight; 104 wks *(percent change compared to control)*					
	M	0%	−11%	−14%	1%	
	F	0%	77%	19%	55%	
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	No immune effects were observed with routine hematology, clinical chemistry, or histopathology evaluations.					
	Doses	0	0.3	1.5	8.0	40
	WBC count; 105 wks *(percent change compared to control)*					
	M	0%	−11%	103%[f]	184%[f]	15%
	F	0%	7%	12%	354%[f]	251%[f]
	Absolute spleen weight; 105 wks *(percent change compared to control)*					
	M	0%	5%	−10%	−32%	−49%
	F	0%	−28%	−44%	−35%	17%
	Relative spleen weight; 105 wks *(percent change compared to control)*					
	M	0%	9%	4%	−29%	−38%
	F	0%	−34%	−45%	−36%	9%
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 spleen weight *(percent change compared to control)*						
	M	0%	–	–	–	18%	13%
	F	0%	–	–	–	−2%	−8%
	Relative spleen weight *(percent change compared to control)*						
	M	0%	–	–	–	24%	14%
	F	0%	–	–	–	−3%	−5%

Reference and study design	Results					
Experiment 2: 0, 40, 60, 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)[d] Diet 13 wks	Doses	0	80	160	320	
	WBC count *(percent change compared to control)*					
	M	0%	−27%	−12%	30%	
	F	0%	−17%	3%	−3%	
	Absolute spleen weight *(percent change compared to control)*					
	M	0%	17%	0%	−17%	
	F	0%	−22%	0%	0%	
	Relative spleen weight *(percent change compared to control)*					
	M	0%	25%	5%	0%	
	F	0%	−12%	0%	−3%	
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
	WBC count *(percent change compared to control)*						
	M	0%	–	–	–	−12%	7%
	F	0%	–	–	–	17%	30%
	Absolute spleen weight *(percent change compared to control)*						
	M	0%	–	–	–	2%	−4%
	F	0%	–	–	–	−10%	−12%*
	Relative spleen weight *(percent change compared to control)*						
	M	0%	–	–	–	5%	5%
	F	0%	–	–	–	−8%	−8%
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 immune effects were observed upon routine histopathology evaluation.						

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	No effects were observed on thymus or spleen histology, RBC or WBC populations, or lymphocyte populations.					
	Doses	0	4	8	10	12	15
	WBC count *(percent change compared to control)*						
	M	0%	−5%	−12%	−7%	1%	−3%
	F	0%	22%	45%	12%	52%	29%
	Absolute spleen weight *(percent change compared to control)*						
	M	0%	−3%	−6%	3%	1%	5%
	F	0%	1%	8%	23%*	17%*	24%*
	Relative spleen weight *(percent change compared to control)*						
	M	0%	3%	4%	7%	−1%	2%
	F	0%	1%	0%	6%	−1%	−2%
	Absolute thymus weight *(percent change compared to control)*						
	M	0%	−1%	3%	−10%	−12%	−25%
	F	0%	−7%	12%	19%	32%	19%
	Relative thymus weight *(percent change compared to control)*						
	M	0%	−1%	3%	−10%	−12%	−25%
	F	0%	−7%	4%	4%	12%	−6%
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[e] 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	Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all of the rats died before the 13-wk necropsy.						
	Doses	0	10	30	100	300	600
	WBC count *(percent change compared to control)*						
	M	0%	4%	7%	15%	–	–
	F	0%	23%*	24%*	62%*	–	–
	Absolute spleen weight *(percent change compared to control)*						
	M	0%	−11%	−16%	−34%	–	–
	F	0%	2%	12%	0%	–	–
	Relative spleen weight *(percent change compared to control)*						
	M	0%	−9%	−12%	−21%	–	–
	F	0%	2%	12%	3%	–	–

Reference and study design	Results				
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	Doses	0	15	25	50
	WBC count *(percent change compared to control)*				
	M	0%	−30%	7%	−6%
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	Doses	0	0.1	1	10
	WBC count *(percent change compared to control)*				
	M	0%	5%	2%	−19%
	F	0%	−2%	24%	6%
	Absolute spleen weight *(percent change compared to control)*				
	M	0%	–	–	123%
	F	0%	–	–	−11%
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
	WBC count *(percent change compared to control)*				
	M	0%	−32%	0%	−3%
	F	0%	−38%	−1%	−41%
Gastrointestinal 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	No GI tract effects were observed as clinical signs or on gross pathology or histopathology examination.				
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	No GI tract effects were observed as clinical signs or on gross pathology or histopathology examination.				

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	No GI tract effects were observed on gross pathology or histopathology examination. Increased salivation and blood stains around the mouth were noted (affected doses and incidences were not reported); it is not clear whether these effects occurred in animals also experiencing convulsions.					
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	Congestion of the GI tract was observed in 50 and 100 mg/kg-d rats that also exhibited mortality (40%) and severe neurotoxicity.					
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	Vomiting was observed more frequently in the 1 and 10 mg/kg-d groups compared to the control or 0.1 mg/kg-d groups, although some episodes occurred during the intubation procedure.					
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	Some nausea and vomiting were reported (incidences and affected dose groups were not reported).					
Hematological 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	Doses	0	1.5	7.0	35	175/100
	RBC count; 105 wks *(percent change compared to control)*					
	M	0%	−4%	3%	−3%	14%
	F	0%	4%	−7%	5%	3%
	Hemoglobin; 105 wks *(percent change compared to control)*					
	M	0%	−6%	3%	−5%	9%
	F	0%	2%	−7%	3%	1%
	Hematocrit; 105 wks *(percent change compared to control)*					
	M	0%	−4%	3%	−4%	9%
	F	0%	3%	−6%	3%	1%
	Platelets; 105 wks *(percent change compared to control)*					
	M	0%	33%	9%	21%	27%

Reference and study design	Results					
	F	0%	−14%	−7%	1%	5%
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	
	RBC count; 104 wks *(percent change compared to control)*					
	M	0%	3%	7%	−2%	
	F	0%	−14%	7%	2%	
	Reticulocyte count; 104 wks *(percent change compared to control)*					
	M	0%	250%	500%*	850%*	
	F	0%	180%*	−40%	20%	
	Hemoglobin; 104 wks *(percent change compared to control)*					
	M	0%	3%	4%	0%	
	F	0%	−1%	1%	−2%	
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
	Hemoglobin levels; 105 wks *(percent change compared to control)*					
	M	0%	6%	6%	3%	−13%
	F	0%	−5%	1%	−9%	−14%
	RBC count; 105 wks *(percent change compared to control)*					
	M	0%	5%	2%	−1%	−9%
	F	0%	−2%	2%	−9%	−13%
	Platelet count; 105 wks *(percent change compared to control)*					
	M	0%	6%	−4%	−10%	−7%
	F	0%	14%	−4%	5%	22%
	Hematocrit; 105 wks *(percent change compared to control)*					
	M	0%	5%	5%	2%	−7%
	F	0%	−5%	0%	−8%	−12%
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)[d] Diet 13 wks	Doses	0	80	160	320	
	RBC count *(percent change compared to control)*					
	M	0%	−5%	−12%*	−2%	
	F	0%	−10%	−1%	1%	
	Reticulocytes *(percent change compared to control)*					
	M	0%	−36%	−13%	15%	
	F	0%	21%	25%	−19%	
	Hematocrit *(percent change compared to control)*					
	M	0%	−1%	−6%	0%	

Reference and study design	Results						
	F	0%	−8%	2%	1%		
	Hemoglobin *(percent change compared to control)*						
	M	0%	−2%	−7%*	−3%		
	F	0%	−5%	4%	1%		
	Platelets *(percent change compared to control)*						
	M	0%	33%	28%	22%		
	F	0%	3%	9%	39%		
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
	RBC count *(percent change compared to control)*						
	M	0%	–	–	–	3%	−1%
	F	0%	–	–	–	−1%	−7%
	Hemoglobin *(percent change compared to control)*						
	M	0%	–	–	–	2%	−1%
	F	0%	–	–	–	−1%	−1%
	Platelet *(percent change compared to control)*						
	M	0%	–	–	–	11%	16%*
	F	0%	–	–	–	−23%	−13%
	Reticulocytes *(percent change compared to control)*						
	M	0%	–	–	–	26%	76%*
	F	0%	–	–	–	−2%	17%
	Hematocrit *(percent change compared to control)*						
	M	0%	–	–	–	3%	0%
	F	0%	–	–	–	0%	−2%
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
	RBC count *(percent change compared to control)*						
	M	0%	1%	−7%	−2%	−4%	−5%
	F	0%	3%	3%	−1%	2%	−2%
	Hemoglobin *(percent change compared to control)*						
	M	0%	−1%	−5%	0%	−1%	−6%
	F	0%	2%	4%	−1	4%	−4%
	Platelet count *(percent change compared to control)*						
	M	0%	21%	11%	13%	−8%	34%
	F	0%	6%	40%	47%	34%	−36%

Reference and study design	Results						
	Hematocrit *(percent change compared to control)*						
	M	0%	2%	−5%	0%	−1%	−4%
	F	0%	3%	4%	0%	4%	−2%
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[e] 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	Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all of the rats died before the 13-wk necropsy.						
	Doses	0	10	30	100	300	600
	Hematocrit *(percent change compared to control)*						
	M	0%	−2%	−1%	−5%	–	–
	F	0%	0%	−4%	−7%	–	–
	Hemoglobin *(percent change compared to control)*						
	M	0%	−3%	−1%	−6%	–	–
	F	0%	0%	−4%	−8%*	–	–
	RBC count *(percent change compared to control)*						
	M	0%	−2%	−2%	−5%	–	–
	F	0%	−1%	−4%	−5%	–	–
	Reticulocytes *(percent change compared to control)*						
	M	0%	−4%	10%	28%	–	–
	F	0%	9%	73%	71%	–	–
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	Doses	0		15	25		50
	RBC count *(percent change compared to control)*						
	M + F	0%		−23%	−12%		−14%
	Hemoglobin *(percent change compared to control)*						
	M + F	0%		−25%	−7%		−11%
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	Doses	0		0.1	1		10
	RBC count *(percent change compared to control)*						
	M	0%		−3%	3%		2%
	F	0%		13%	7%		11%
	Reticulocyte count *(percent change compared to control)*						
	M	0%		−66%	0%		−50%
	F	0%		−17%	−50%		0%
	Hematocrit *(percent change compared to control)*						
	M	0%		−4%	2%		0%
	F	0%		6%	1%		7%

Reference and study design	Results				
	Hemoglobin *(percent change compared to control)*				
	M	0%	5%	−2%	0%
	F	0%	8%	−2%	8%
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	Histopathological examination revealed increased numbers of degenerate or necrotic megakaryocytes in all bone marrow sections.				
	Doses	0	0.1	1	10
	RBC count *(percent change compared to control)*				
	M	0%	−3%	2%	−3%
	F	0%	0%	−1%	2%
	Reticulocyte count *(percent change compared to control)*				
	M	0%	−33%	−50%	−50%
	F	0%	−18%	−36%	45%
	Hematocrit *(percent change compared to control)*				
	M	0%	−7%	−4%	−1%
	F	0%	10%	7%	3%
	Hemoglobin *(percent change compared to control)*				
	M	0%	−10%	−8%	−6%
	F	0%	6%	6%	3%

*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]No musculoskeletal evidence is presented in this table as no animal study reported effects on the musculoskeletal system and all human effects were in case reports (see summary in Appendix C, Section C.2).
[b]Incidence counts exclude individuals from which blood was obtained via the orbital sinus.
[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]Doses were calculated by the study authors.
[e]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.
[f]Standard deviations accompanying the mean response in a given dose group were high, suggesting uncertainty in the accuracy of the reported percent change compared to control.

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

C.3.3. Genotoxicity

RDX

RDX has tested negative in a variety of in vitro tests for genotoxicity, including mutation assays in multiple strains of *Salmonella typhimurium* (with or without metabolic activation), recombination in *Saccharomyces cerevisiae* strain D3, and forward mutations in both V79 Chinese hamster lung cells and mouse lymphoma L5178Y cells. However, in genotoxicity assays designed to be more sensitive, RDX did show some positive results. For example, when the concentration of S9 was doubled, the mutagenicity of RDX was about twice that of background. RDX also showed positive mutagenic results with metabolic activation in a chemiluminescent assay (Mutatox assay). In some cases, the interpretation of testing data for RDX was complicated by the tendency of the compound to precipitate out of DMSO solution (the usual vehicle) at concentrations ≥250 µg/mL (Reddy et al., 2005). As with other studies of RDX, the purity of the test compound was unknown in several (particularly older) studies. A summary of the results of in vitro genotoxicity studies of RDX is presented in Table C-13.

RDX has produced negative results in all reverse mutation assays in *S. typhimurium* that use standard levels of the metabolic activation system (S9). No evidence of reverse mutation was observed in *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), either with or without the addition of S9 metabolic activating mixture (Neuwoehner et al., 2007; George et al., 2001; Lachance et al., 1999; Tan et al., 1992; Cholakis et al., 1980; Whong et al., 1980; Cotruvo et al., 1977; Simmon et al., 1977). One exception is a finding of weak mutagenic activity of RDX to *S. typhimurium* strain TA97a (mutagenicity index = 1.5−2.0) (Pan et al., 2007a). However, this assay used a high percentage of S9 fraction (9% instead of 4%), indicating that extensive metabolic activation is needed to elicit a mutagenic response.

RDX did not cause gene recombination in *S. cerevisiae* strain D3 at concentrations up to 23 µg/mL, with or without metabolic activation (Cotruvo et al., 1977; Simmon et al., 1977). Simmon et al. (1977) noted that the negative findings should be considered in the context of the low concentrations tested. RDX was negative in assays with *S. choleraesius* and *E. coli* with and without metabolic activation (Neuwoehner et al., 2007). Similarly, RDX did not induce forward mutations (HGPRT locus) in V79 Chinese hamster lung cells, with or without metabolic activation, although minimal cytotoxicity was observed at 180 µM (Lachance et al., 1999). However, RDX produced revertants in two of three trials in the Mutatox assay with the bacterium *Vibrio fisheri* when tested at doses up to 2.5 µg/tube, with and without S9 (Arfsten et al., 1994). In the presence of S9, a dose-response was observed; in the absence of S9, no dose-response relationship was detected (Arfsten et al., 1994). RDX did not induce forward mutations in mouse lymphoma L5178Y cells with or without metabolic activation (Reddy et al., 2005). During an accompanying range-finding study, precipitates occurred at doses ≥250 µg/mL, suggesting that concentrations of RDX in DMSO reported beyond 250 µg/mL may not be accurate.

Table C-13. Summary of in vitro studies of the genotoxicity of RDX

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1,000 µg/plate	–	–	Metabolic activation with S9	Cholakis et al. (1980)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538 TA100, TA98	14 µg/plate	–	–	Effect of disinfection treatments on mutagenicity tested: RDX was not mutagenic in any strain before or after disinfection treatment with chlorine or ozone	Simmon et al. (1977)
Reverse mutation	*S. typhimurium* TA98, TA100	250 µg/plate	–	–	Study authors noted that results were consistent with literature	George et al. (2001)
Reverse mutation	*S. typhimurium* TA98, TA100	1 mg/plate	–	–	Metabolic activation with S9	Tan et al. (1992)
Reverse mutation	*S. typhimurium* TA98, TA100	1,090 µg/plate	–	–	High S9 activation (9%) used	Pan et al. (2007a)
Reverse mutation	*S. typhimurium* TA97a	32.7 µg/plate	–	±	High S9 activation (9%) used; study authors concluded that RDX "required intensive metabolic activation" to exhibit mutagenicity in this strain	Pan et al. (2007a)
Reverse mutation	*S. typhimurium TA1535, TA1537, TA1538 TA100, TA98*	Up to 2.5 mg/plate		–	Results were reported qualitatively only; quantitative results were not presented. Not clear if assay was also performed without S9	Whong et al. (1980)
Reverse mutation	*Vibrio fischeri*	0.004 µg/tube	±	+	Mutatox assay with metabolic activation (S9)	Arfsten et al. (1994)

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Reverse mutation (*umu* test)	*Salmonella choleraesius subsp. chol.* (prior *S. typhimurium*) TA1535/pSK1002	20.6 µg/mL	–	–	No observed effect concentration; tested at highest concentration where the induction rate was below 1.5 for the first time and the growth factor was below 0.5	Neuwoehner et al. (2007)
Reverse mutation (NM2009 test)	*S. choleraesius subsp. chol.* NM2009, TA1535/pSK1002/pNM12	20.6 µg/mL	–	–	No observed effect concentration; tested at highest concentration where the induction rate was below 1.5 for the first time and the growth factor was below 0.5	Neuwoehner et al. (2007)
Induction of the *sfiA* gene (SOS chromotest)	*Escherichia coli* PQ37	20.6 µg/mL	–	–	No observed effect concentration; tested at highest concentration where the induction rate was below 1.5 for the first time and the growth factor was below 0.5	Neuwoehner et al. (2007)
Reverse mutation	*S. typhimurium*, TA98, TA100	24.8 µg/mL	–	–	No observed effect concentration; metabolic activation with S9	Neuwoehner et al. (2007)
Reverse mutation	*S. typhimurium* TA98, TA100	2.6 µg/mL	–	–	No observed effect concentration; metabolic activation with S9; minimal cytotoxicity was observed at 180 µM	Lachance et al. (1999)
Reverse mutation	*S. typhimurium* TA1535, TA1536, TA1537, TA1538 TA100, TA98	30.8 µg/mL	–	–	Metabolic activation with S9	Cotruvo et al. (1977)
Genotoxicity studies in nonmammalian eukaryotic organisms						
Recombination induction	*Saccharomyces cerevisiae* D3	23 µg/mL	–	–	Study authors concluded that this microorganism did not appear to be useful for detecting mutagenicity in several compounds tested	Simmon et al. (1977)
Recombination induction	*S. cerevisiae* D3	30.8 µg/mL	–	–	Metabolic activation with S9	Cotruvo et al. (1977)

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in mammalian cells						
Forward mutation	Chinese hamster lung fibroblasts V79 cells	40 µg/mL	–	–	Minimal cytotoxicity observed at 40 µg/mL (limit of solubility)	Lachance et al. (1999)
Mutation	L5178Y mouse lymphoma cells	500 µg/mL	–	–	No or low cytotoxicity seen at these concentrations; however, precipitate was observed at >250 µg/mL	Reddy et al. (2005)
Unscheduled DNA synthesis; DNA repair	WI-38 cells, human diploid fibroblasts	4,000 µg/mL	–	–	Precipitates were observed at concentrations of RDX ≥40 µg/mL	Dilley et al. (1979)

[a]Lowest effective dose for positive results; highest dose tested for negative results.
[b]+ = positive; ± = equivocal or weakly positive; – = negative.

RDX did not induce unscheduled DNA synthesis, with or without metabolic activation, using human diploid fibroblasts (WI-38 cells) when tested in DMSO at concentrations up to 4,000 µg/mg; however, precipitation of RDX at high concentrations in cell culture media makes interpretation of these results difficult (Dilley et al., 1979). Only two in vivo studies are available for the genotoxicity of RDX; these are summarized in Table C-14. RDX did not decrease the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs), nor did it induce micronucleated PCEs in an in vivo mouse bone marrow micronucleus assay in young adult male CD-1 mice (Reddy et al., 2005). RDX was considered negative for the induction of dominant lethal mutations in male CD rats fed RDX at nominal doses from 0 to 50 mg/kg-day for 15 weeks prior to mating with untreated virgin females (Cholakis et al., 1980). Females sacrificed at midgestation showed no statistically significant effects on number of corpora lutea, implants, or live or dead embryos (Cholakis et al., 1980).

Metabolites of RDX

Several metabolites of RDX, N-nitroso derivatives of the parent compound (mononitroso, dinitroso, and trinitroso compounds, abbreviated MNX, DNX, and TNX, respectively) (Musick et al., 2010; Major et al., 2007) have been tested directly for genotoxicity (Pan et al., 2007a; George et al., 2001; Snodgrass, 1984). Miniature pigs were used to detect these trace metabolites because the swine model of the GI tract more closely resembles that of humans (Major et al., 2007); an identification and quantification of RDX metabolites in humans has not been conducted. A summary of the results of in vitro and in vivo genotoxicity studies of metabolites of RDX is presented in Table C-15.

Pan et al. (2007a) studied the mutagenicity of two metabolites, MNX and TNX. These metabolites were not mutagenic in *S. typhimurium* strain TA97a at normal levels of S9, but were clearly mutagenic at enhanced concentrations of S9 (4% versus 9% S9). The observation that these metabolites were positive in *S. typhimurium* strain TA97a is likely due to this strain's higher sensitivity for frameshift mutations that occur at a cluster of cytosine residues in the mutated gene for histidine synthesis in this strain (Pan et al., 2007a). These metabolites were also weakly mutagenic in *S. typhimurium* strain TA102, again with high levels of S9. Strain TA102 was developed with an A:T base pair at the site of mutation and its sensitivity was increased by the addition of some 30 copies of a plasmid containing the mutant gene that is available for back mutation. This strain is sensitive to many oxidative mutagenic compounds (Levin et al., 1982). Other metabolites with potential human relevance identified in the urine of miniature pigs have not been assessed for their genotoxicity (Major et al., 2007). In assays with *S. typhimurium* strains TA98 and TA100, TNX was positive in strain TA100 with and without S9, but not in strain TA98; MNX and DNX were not mutagenic in either strain (George et al., 2001).

1 **Table C-14. Summary of in vivo studies of the genotoxicity of RDX**

Endpoint	Test system	Dose/ concentration	Results	Comments	Reference
In vivo genotoxicity studies in mammalian systems					
Micronucleus formation	CD-1 mouse bone marrow	Single dose of 62.5, 125, or 250 mg/kg	No significant decrease in PCE:NCE ratios; no induction of micronucleated PCE at any dose	250 mg/kg was maximum tolerated dose determined in dose range-finding study	Reddy et al. (2005)
Dominant lethal mutations	Male CD rats dosed and mated with untreated female rats	0, 5, 16, or 50 mg/kg-d for 15 wk	No statistically or biologically significant effects on fertility; determined to be negative for the induction of lethal mutations	Males in the high-dose group experienced lower food consumption and weight gain compared with all other groups	Cholakis et al. (1980)

2

Table C-15. Summary of in vitro and in vivo studies of the genotoxicity of RDX metabolites

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA97a, TA102	22 µg/plate	–	+	Mono and trinitroso metabolites (MNX and TNX); high S9 activation (9%) used	Pan et al. (2007a)
Reverse mutation	*S. typhimurium* TA98, TA100	500 µg/plate	+	+	Positive in TA100 (but not in TA98) only for TNX; MNX and DNX were negative	George et al. (2001)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100	NR	–	–	Mononitroso metabolite, MNX; metabolic activation with S9	Snodgrass (1984)
Genotoxicity studies in mammalian cells—in vitro						
Forward mutation	Mouse lymphoma thymidine kinase	NR	+	+	Mononitroso metabolite, MNX; metabolic activation with S9	Snodgrass (1984)
Chromosomal aberrations	Chinese hamster ovary cells	NR	–	+	Mononitroso metabolite, MNX; metabolic activation with S9	Snodgrass (1984)
Unscheduled DNA synthesis; DNA repair	Primary rat hepatocytes	NR	+	ND	Mononitroso metabolite, MNX; additional metabolic activation not required with S9	Snodgrass (1984)
In vivo genotoxicity studies in mammalian systems						
Dominant lethal mutations	Male mice dosed and mated with untreated female mice	NR	–	ND	Mononitroso metabolite, MNX; additional metabolic activation not required with S9	Snodgrass (1984)

[a]Lowest effective dose for positive results; highest dose tested for negative results; NR = not reported.
[b]+ = positive; ± = equivocal or weakly positive; – = negative; ND = not determined.

The genotoxicity of MNX was positive in three out of five assays conducted for the U.S. Army (Snodgrass, 1984). MNX was positive with or without metabolic activation in the mouse lymphoma forward mutation assay at the thymidine kinase locus, for chromosomal aberrations in Chinese hamster ovary cells, and in the primary rat hepatocyte unscheduled DNA synthesis assay. MNX was not considered positive in *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), either with or without the addition of S9 metabolic activating mixture or in an in vivo dominant lethal mutation assay in mice. However, this study is of limited use due to a significant lack of details including information on dosing, raw data, and statistical reporting.

In summary, RDX is not mutagenic or genotoxic in vitro or in vivo in typical assays used to detect genotoxicity. In two in vitro studies using more sensitive assays and conditions for detecting mutagenicity, RDX was found to be positive. Several studies showed that the N-nitroso metabolites are genotoxic, but the formation and quantification of these metabolites in humans is not known.

APPENDIX D. DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES

This appendix provides technical detail on dose-response evaluation and determination of points of departure (POD) for relevant toxicological endpoints. The endpoints were modeled using the U.S. Environmental Protection Agency (EPA) Benchmark Dose Software (BMDS, Versions 2.4). Sections D.1 (noncancer) and D.2 (cancer) describe the common practices used in evaluating the model fit and selecting the appropriate model for determining the POD, as outlined in the *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b). In some cases, it may be appropriate to use alternative methods, based on statistical judgement; exceptions are noted as necessary in the summary of the modeling results.

D.1. BENCHMARK DOSE MODELING SUMMARY FOR NONCANCER ENDPOINTS

The noncancer endpoints that were selected for dose-response modeling are presented in Table D-1. For each endpoint, the doses and response data used for the modeling are presented.

Table D-1. Noncancer endpoints selected for dose-response modeling for RDX

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)
Convulsions Crouse et al. (2006)[a]	Female F344 rat	0 4 8 10 12 15	0/10 (0%) 0/10 (0%) 2/10 (20%) 3/10 (30%) 5/10 (50%) 5/10 (50%)
	Male F344 rat	0 4 8 10 12 15	0/10 (0%) 0/10 (0%) 1/10 (10%) 3/10 (30%) 8/10 (80%) 7/10 (70%)

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)
	Male and female F344 rat, combined	0 4 8 10 12 15	0/20 (0%) 0/20 (0%) 3/20 (15%) 6/20 (30%) 13/20 (65%) 12/20 (60%)
Convulsions Cholakis et al. (1980)	Female F344 rat (gestational exposure)	0 0.2 2 20	0/24 (0%) 0/24 (0%) 1/24 (4%) 18/24 (75%)
Testicular degeneration Lish et al. (1984)	Male B6C3F$_1$ mouse	0 1.5 7 35 107	0/63 (0%) 2/60 (3%) 2/62 (3%) 6/59 (10%) 3/27 (11%)
Prostate suppurative inflammation Levine et al. (1983)	Male F344 rat	0 0.3 1.5 8 40	2/54 (4%) 4/55 (7%) 9/52 (17%) 12/55 (22%) 19/31 (61%)

[a]For convulsions in Crouse et al. (2006), the incidence rates across doses were determined to be not statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for this endpoint prior to modeling.

In addition to the endpoints presented in Table D-1, the combined incidence of seizure and mortality was modeled for Crouse et al. (2006) to determine the effect of possible underestimation of seizures, as discussed in Section 2.1.6. Table D-2 presents the data on this combined incidence.

Table D-2. Convulsion or mortality endpoints from Crouse et al. (2006) selected for dose-response modeling for RDX

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)
Convulsion or mortality Johnson (2015)	Female F344 rat	0 4 8 10 12 15	0/10 (0%) 0/10 (0%) 3/10 (30%) 5/10 (50%) 9/10 (90%) 8/10 (80%)

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)
	Male F344 rat	0	0/10 (0%)
		4	0/10 (0%)
		8	2/10 (20%)
		10	4/10 (40%)
		12	8/10 (80%)
		15	7/10 (70%)
	Male and female F344 rat, combined	0	0/20 (0%)
		4	0/20 (0%)
		8	5/20 (25%)
		10	9/20 (45%)
		12	17/20 (85%)
		15	15/20 (75%)

[a]Incidence was defined for each animal as the presence of convulsion or mortality. The incidence rates across doses for this endpoint were determined to be not statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for this endpoint prior to modeling.

D.1.1. Evaluation of Model Fit and Model Selection

For each dichotomous endpoint, BMDS dichotomous models[5] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the benchmark response (BMR).

From among the models exhibiting adequate fit, the best-fit model was selected for estimation of the BMD. This model selection was conducted in two stage, first from among only the multistage models to determine a representative multistage model, and second from among the representative multistage model and the non-multistage models. In each stage, the BMDL estimates (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC values of the models considered in that stage were used to make the selection, as follows. If the BMDL estimates were "sufficiently close," that is, differed by threefold or less, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the model with the lowest BMDL was selected. The model selected in the second stage was considered the best-fit model.

The BMDL estimate (95% lower confidence limit on the benchmark dose [BMD], as estimated by the profile likelihood method) and Akaike's information criterion (AIC) value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL

[5]Unless otherwise specified, all available BMDS dichotomous models besides the alternative and nested dichotomous models were fitted. The following parameter restrictions were applied: for the Log-Logistic model, restrict slope ≥1; for the Gamma and Weibull models, restrict power ≥1.

estimates were "sufficiently close" (i.e., differed by at most threefold), the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

D.1.2. Modeling Results

The tables that follow summarize the modeling results for the noncancer endpoints modeled.

Nervous System Effects

Tables D-3 to D-5 (and Figures D-1 to D-3) present the BMD modeling results for incidence of convulsions for female, male, and male and female F344 rats combined based on data from Crouse et al. (2006), using BMRs of 10, 5, and 1% extra risk (ER). Table D-6 (and Figure D-4) present the BMD modeling results for incidence of convulsions for female F344 rats based on data from Cholakis et al. (1980), using BMRs of 10, 5, and 1% ER. Table D-7 (and Figure D-5) presents the BMD modeling results for combined incidence of convulsions and mortality for male and female rats combined based on data from Crouse et al. (2006).

Table D-3. Model predictions for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.923	55.085	3.10	0.355	The Quantal-Linear model had a BMD more than 10 times lower than the lowest dose, and the residual at the lowest dose was modereatly high (−1.3). Thus, this model was excluded from consideration. Of the higher degree multistage models, the multistage 2° model was selected as the representative multistage model based on lowest AIC. From among the multistage 2° and non-multistage models, the multistage 5° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.733	56.607	1.60	0.681	
LogLogistic	0.929	55.076	2.87	0.468	
Probit	0.793	56.086	1.86	0.649	
LogProbit	0.952	54.798	3.63	0.919	
Weibull	0.892	55.420	2.30	0.259	
Multistage 2°	**0.954**	**53.595**	**1.69**	**0.236**	
Quantal-Linear	0.733	56.131	0.263	0.176	
Multistage 3°	0.885	55.525	1.99	0.238	
Multistage 4°	0.885	55.525	1.99	0.236	
Multistage 5°	0.885	55.525	1.99	0.235	
Dichotomous-Hill	0.964	56.265	4.77	0.778	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, −0.67, 0.14, 0.11, 0.64, and −0.51, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 5.46 and 2.47 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 3.81 and 1.21 mg/kg-day, respectively.

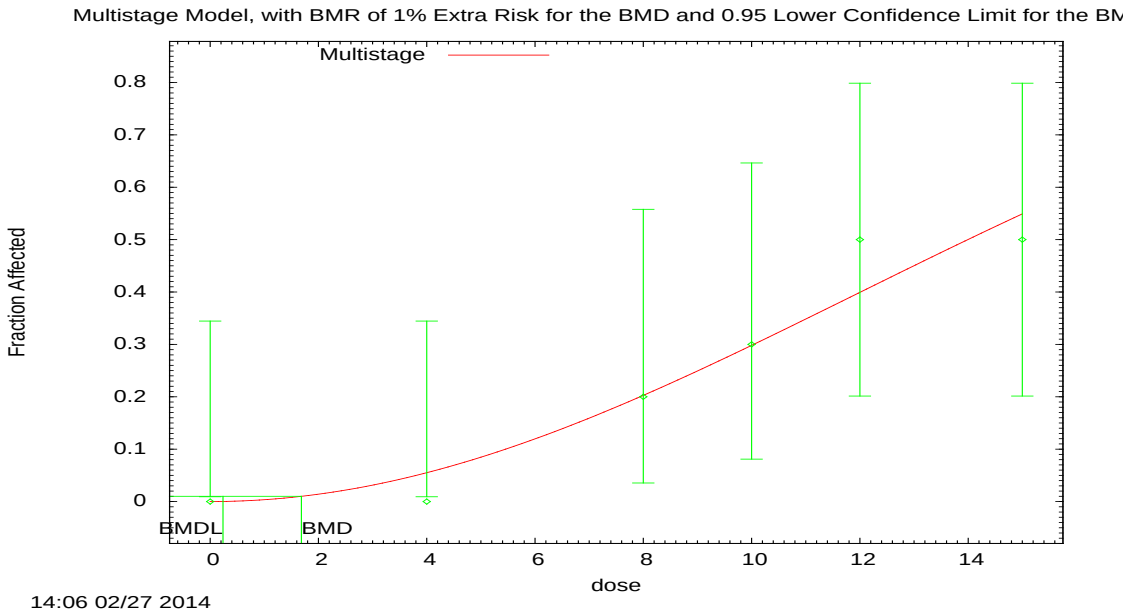


Figure D-1. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% Extra risk

BMD = 1.68508

BMDL at the 95% confidence level = 0.236479

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.0172961
Beta(2)	0.00353947	0.002476

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−24.9756	6			
Fitted model	−25.7976	1	1.64388	5	0.8959
Reduced model	−33.7401	1	17.529	5	0.003598

AIC: = 53.5951

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.0551	0.551	0	10	−0.763
8	0.2027	2.027	2	10	−0.021
10	0.2981	2.981	3	10	0.013
12	0.3993	3.993	5	10	0.65
15	0.549	5.49	5	10	−0.312

Chi^2 = 1.1 d.f. = 5 *p*-value = 0.9538

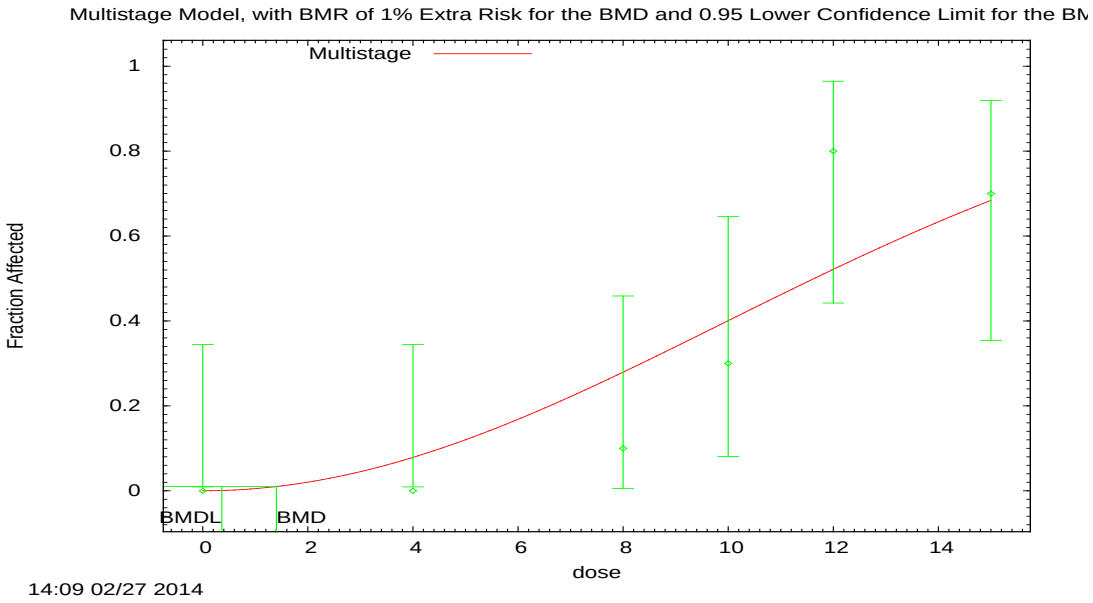
Chi^2 = 1.16 d.f

Table D-4. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.482	48.534	4.96	2.32	Of the multistage models that provided an adequate fit, the multistage 2° model was selected based on lowest AIC. From among the multistage 2° and non-multistage models, the multistage 2° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.335	49.692	2.86	0.975	
LogLogistic	0.522	48.248	4.79	2.38	
Probit	0.363	49.460	3.60	1.01	
LogProbit	0.530	48.224	5.41	3.00	
Weibull	0.376	49.496	3.52	1.43	
Multistage 2°	**0.307**	**50.335**	**1.40**	**0.363**	
Quantal-Linear	0.0553	56.530	0.189	0.131	
Multistage 5°[b]	0.361	49.607	3.42	0.392	
Multistage 4°[c]	0.361	49.607	3.42	0.392	
Multistage 3°	0.515	47.803	2.82	0.457	
Dichotomous-Hill	0.701	48.408	6.64	3.47	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, −0.92, −1.26, −0.65, 1.76, and 0.11, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 4.54 and 2.95 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 3.17 and 1.63 mg/kg-day, respectively.

[b]The Multistage 5° model may appear equivalent to the Multistage 4° model; however, differences exist in digits not displayed in the table.



BMR = 1% ER; dose shown in mg/kg-day.

Figure D-2. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% ER

BMD = 1.40125

BMDL at the 95% confidence level = 0.363499

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0
Beta(2)	0.00511858	0.00691555

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−20.4721	6			
Fitted model	−24.1672	1	7.39017	5	0.1932
Reduced model	−37.4599	1	33.9755	5	<0.0001

AIC: = 50.3345

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.0786	0.786	0	10	−0.924
8	0.2793	2.793	1	10	−1.264
10	0.4006	4.006	3	10	−0.649
12	0.5215	5.215	8	10	1.763
15	0.6839	6.839	7	10	0.11

Chi^2 = 5.99 d.f. = 5 *p*-value = 0.3069

Table D-5. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.484	101.79	4.02	2.03	The Quantal-Linear model did not fit the data adequately (goodness-of-fit *p*-value <0.10), so it was excluded from consideration. Of the higher degree multistage models, the Multistage 3° model was selected based on lowest AIC. From among the multistage 3° and non-multistage models, the multistage 3° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.231	104.55	2.04	0.987	
LogLogistic	0.512	101.66	3.79	2.00	
Probit	0.291	103.61	2.57	1.03	
LogProbit	0.557	101.25	4.50	2.69	
Weibull	0.369	102.91	2.94	1.35	
Multistage 2°	0.364	103.03	1.53	0.544	
Quantal-Linear	0.0369	111.56	0.222	0.169	
Multistage 5°[b] Multistage 4°	0.502	100.91	3.02	0.549	
Multistage 3°	**0.502**	**100.91**	**3.02**	**0.569**	
Dichotomous-Hill	0.696	101.64	5.62	2.90	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, −0.69, −0.25, −0.06, 1.62, and −1.08, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 6.60 and 4.59 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 5.19 and 2.66 mg/kg-day, respectively.

[b]For the Multistage 5° model, the beta coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 4° model.

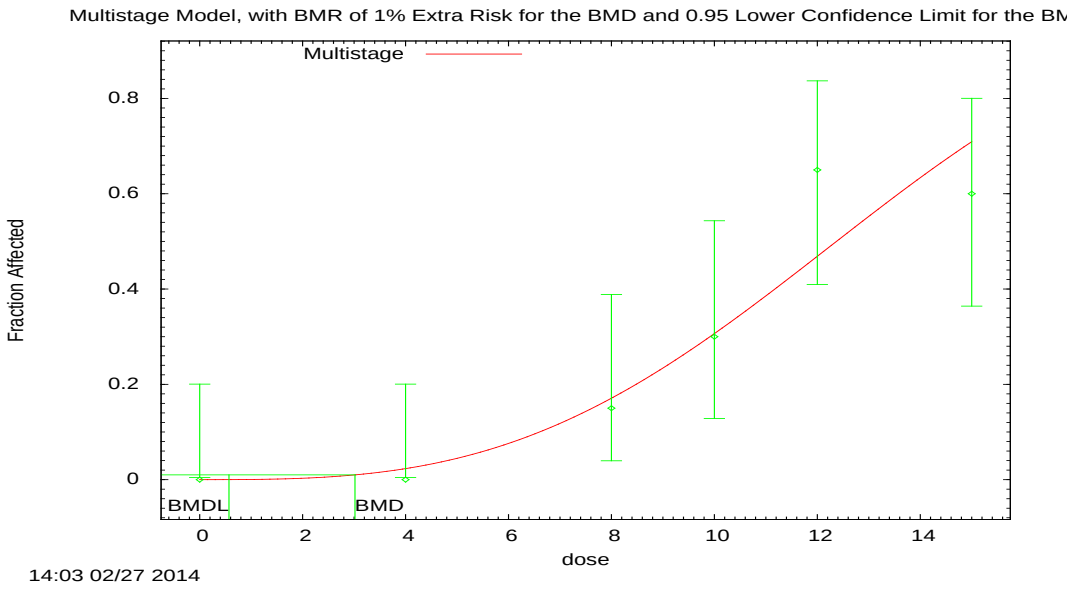


Figure D-3. Plot of incidence rate by dose, with the fitted curve of the multistage 3° model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% ER; dose shown in mg/kg-day.

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% Extra risk

BMD = 3.01676

BMDL at the 95% confidence level = 0.569284

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.00163806
Beta(2)	0	0.00485933
Beta(3)	0.000366065	0

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−47.0806	6			
Fitted model	−49.4567	1	4.75213	5	0.4469
Reduced model	−71.5289	1	48.8965	5	<0.0001

AIC: = 100.913

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.0232	0.463	0	20	−0.689
8	0.1709	3.418	3	20	−0.248
10	0.3065	6131	6	20	−0.063
12	0.4688	9.375	13	20	1.624
15	0.7093	14.186	12	20	−1.076

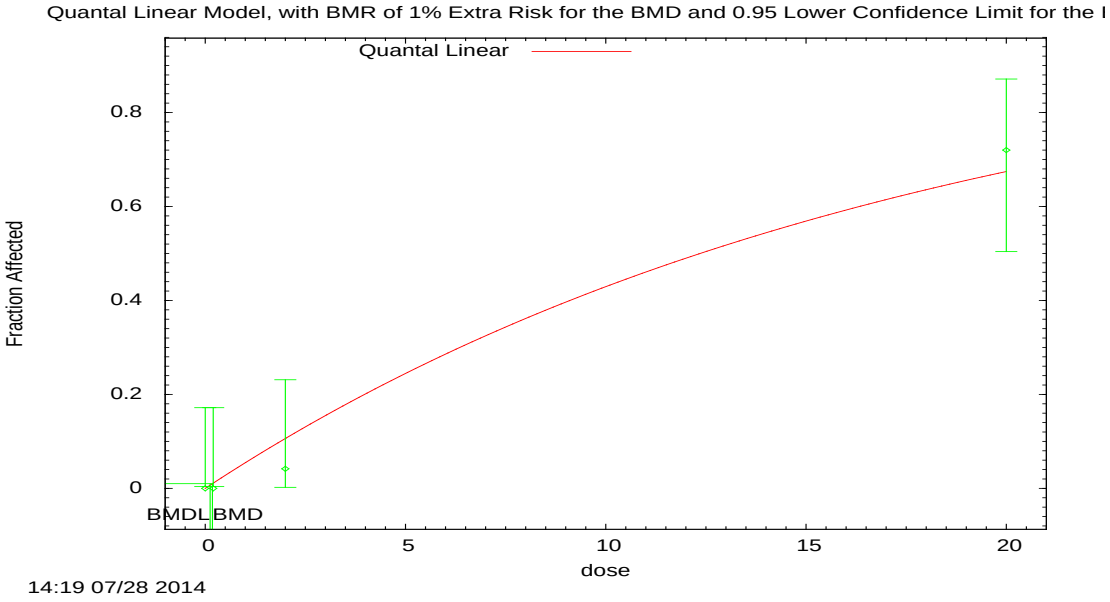
Chi^2 = 4.34 d.f. = 5 *p*-value = 0.5021

Table D-6. Model predictions for convulsions in female F344 rats exposed to RDX by gavage on GDs 6–19 (Cholakis et al., 1980); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.989	42.003	0.866	0.149	Of the multistage models, the quantal-linear model was selected based on lowest AIC. From among the quantal-linear and non-multistage models, the quantal-linear model is selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.526	43.556	2.46	1.05	
LogLogistic	0.991	41.996	0.902	0.201	
Probit	0.577	43.348	1.96	0.871	
LogProbit	1.000	41.963	1.11	0.335	
Weibull	0.983	42.026	0.798	0.148	
Multistage 3°[b]	0.960	42.113	0.638	0.146	
Multistage 2°[c]	0.960	42.113	0.638	0.146	
Quantal-Linear	**0.669**	**42.077**	**0.179**	**0.123**	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-day were 0.00, −0.52, −1.03, and 0.49, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 1.88 and 1.29 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 0.915 and 0.628 mg/kg-day, respectively.

[b]The Multistage 3° model may appear equivalent to the Multistage 2° model; however, differences exist in digits not displayed in the table.



BMR = 1% ER; dose shown in mg/kg-day.

Figure D-4. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in female F344 rats exposed to RDX by gavage on GDs 6–19 (Cholakis et al., 1980).

Quantal Linear Model using Weibull Model (Version: 2.16; Date: 2/28/2013)
The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-slope*dose)]

Benchmark Dose Computation
BMR = 1% ER
BMD = 0.179224
BMDL at the 95% confidence level = 0.122966

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0.0384615
Slope	0.056077	0.0588587
Power	Not applicable	1

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−18.9808	4			
Fitted model	−20.0384	1	2.11537	3	0.5488
Reduced model	−47.9793	1	57.9972	3	<0.0001

AIC: = 42.0769

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	24	0
0.2	0.0112	0.268	0	24	−0.52
2	0.1061	2.546	1	24	−1.025
20	0.6742	16.856	18	25	0.488

Chi^2 = 1.56 d.f. = 3 *p*-value = 0.6686

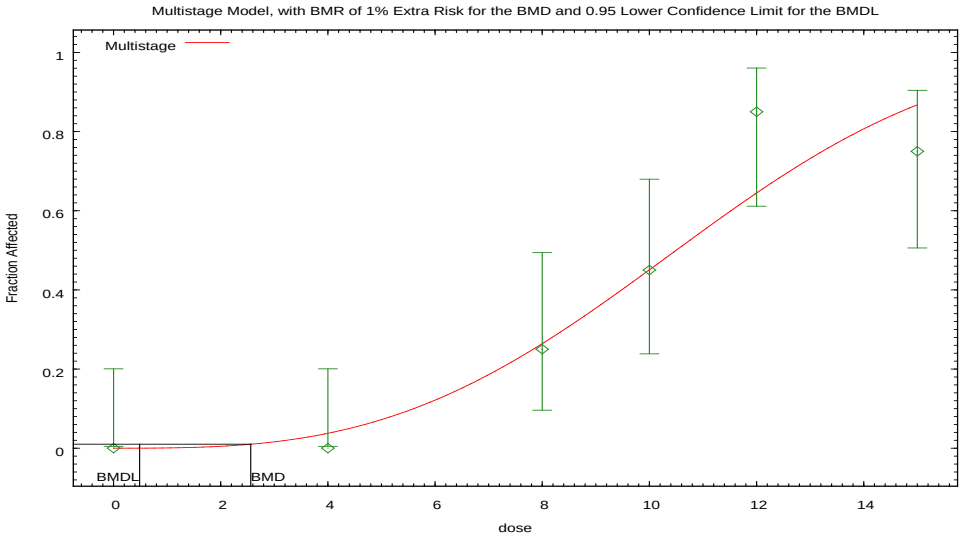
Table D-7. Model predictions for combined incidence of convulsion and mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.245	99.260	3.73	2.10	The log-logistic and quantal-linear models did not achieve an adequate fit (goodness-of-fit *p*-value <0.10). The multistage 2° model was excluded from model selection because the residual in the lowest dose group, near the BMD, was above 1.5 in absolute value. Of the remaining multistage models, the multistage 3° model was selected based on lowest AIC. From among the multistage 3° and non-multistage models, the multistage 3° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Dichotomous-Hill	0.436	98.317	5.22	3.04	
Logistic	0.0859	102.17	1.81	0.846	
LogLogistic	0.305	98.593	3.70	2.20	
Probit	0.101	101.85	2.16	0.853	
LogProbit	0.316	98.465	4.22	2.75	
Weibull	0.152	101.16	2.45	1.24	
Multistage 4°[b]	0.229	99.182	2.56	0.486	
Multistage 3°[c]	**0.229**	**99.182**	**2.56**	**0.486**	
Multistage 2°	0.165	102.01	1.22	0.470	
Quantal-Linear	0.0052	113.90	0.144	0.113	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0, −0.88, −0.14, −0.01, 1.92, and −1.55, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 5.60 and 3.85 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 4.41 and 2.25 mg/kg-day, respectively.

[b]The Multistage 4° model may appear equivalent to the Multistage 3° model; however, differences exist in digits not displayed in the table.

[c]The Multistage 3° model may appear equivalent to the Multistage 4° model; however, differences exist in digits not displayed in the table.



BMR = 1% ER; dose shown in mg/kg-day.

Figure D-5. Plot of incidence rate by dose with fitted curve for Multistage 3° model for model predictions for combined incidence of convulsion and mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).

Multistage Model (Version: 3.4; Date: 05/02/2014)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% Extra risk

BMD = 2.56012

BMDL at the 95% confidence level = 0.486284

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.0272036
Beta(2)	0	0.00626035
Beta(3)	0.000598962	0

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−44.71	6			
Fitted model	−48.59	1	7.76102	5	0.17
Reduced model	−9.88	1	70.3406	5	<0.0001

AIC: = 99.1817

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.0376	0.752	0	20	−0.88
8	0.2641	5.282	5	20	−0.14
10	0.4506	9.012	9	20	−0.01
12	0.6448	12.896	17	20	1.92
15	0.8675	17.351	15	20	−1.55

Chi^2 = 6.88 d.f. = 5 *p*-value = 0.2294

Male Reproductive Effects

Table D-8 (and Figure D-6) presents the BMD modeling results for incidence of testicular degeneration for male B6C3F$_1$ mice based on data from Lish et al. (1984), using a BMR of 10% ER.

Table D-8. Model predictions for testicular degeneration in male B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma[b] Weibull Quantal-Linear	0.357	101.10	66.5	35.4	The Log-Probit model was selected based on lowest BMDL. (BMDLs differed by more than threefold. The multistage models had the same AIC values and BMDLs, so selection of a
Logistic	0.159	103.40	97.1	66.1	
LogLogistic	0.377	100.91	63.6	32.3	
Probit	0.178	103.12	93.1	61.4	

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
LogProbit	**0.876**	**97.564**	**56.0**	**16.3**	representative multistage model was unnecessary.)
Multistage 2°[c] Multistage 3° Multistage 4°	0.357	101.10	66.5	35.4	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were 0.00, 0.32, −0.61, 0.43, and −0.17, respectively. The BMD_{05} and $BMDL_{05}$ values for the selected model were 7.42 and 0.0477 mg/kg-day, respectively; the BMD_{01} and $BMDL_{01}$ values for the selected model were 0.168 and 2.83×10^{-13} mg/kg-day, respectively.

[b]For the Gamma and Weibull models, the power parameter estimates were 1 (boundary of parameter space). The models in this row are equivalent to the Quantal-Linear model.

[c]The Multistage 3° and 4° model had b3 and b4 coefficient estimates of 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model. The models in this row may appear equivalent to the Gamma model; however, differences exist in digits not displayed in the table.

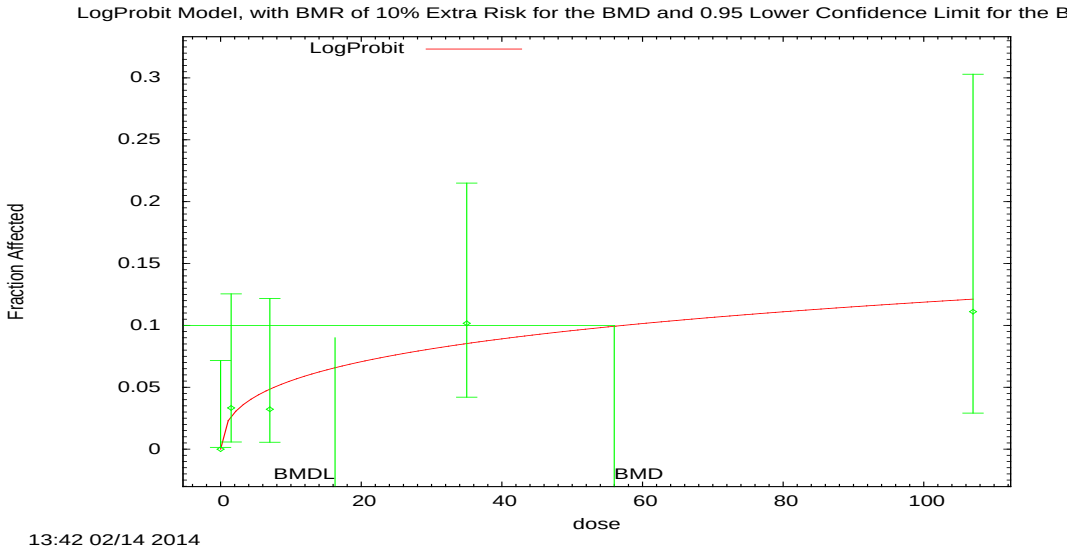


Figure D-6. Plot of incidence rate by dose, with fitted curve for selected model, for testicular degeneration in male B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984).

Probit Model (Version: 3.3; Date: 2/28/2013)

The form of the probability function is: P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)), where CumNorm(.) is the cumulative normal distribution function

Slope parameter is not restricted

Benchmark Dose Computation

BMR = 10% ER

BMD = 55.9784

BMDL at the 95% confidence level = 16.2787

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Intercept	−2.0054E+00	−1.9976E+00
Slope	0.179828	0.172286

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−46.4212	5			
Fitted model	−46.7817	2	0.721088	3	0.8682
Reduced model	−52.1663	1	11.4902	4	0.02157

AIC: = 97.5635

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	63	0
1.5	0.0267	1.599	2	60	0.321
7	0.0489	3.033	2	62	−0.608
35	0.086	5.072	6	59	0.431
107	0.122	3.294	3	27	−0.173

Chi^2 = 0.69 d.f. = 3 *p*-value = 0.8759

Kidney/Urogenital System Effects

Table D-9 (and Figure D-7) presents the BMD model results for incidence of suppurative inflammation of the prostate in male F344 rats based on data from Levine et al. (1983), using a BMR of 10% ER.

Table D-9. Model predictions for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma[b] Multistage 2° Quantal-Linear Multistage 3° Multistage 4°	0.288	200.37	4.61	3.24	The Log-Probit model is selected based on lowest BMDL. (BMDLs differ by more than threefold. The multistage models had the same AIC values and BMDLs, so selection from among the multistage models was unnecessary.)
Logistic	0.102	203.50	10.8	8.58	
LogLogistic	0.328	200.05	3.33	2.09	
Probit	0.116	203.10	9.91	7.96	
LogProbit	**0.204**	**202.03**	**1.67**	**0.469**	
Weibull[g]	0.288	200.37	4.61	3.24	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were −0.289, 0.172, 0.846, −1.298, and 0.819, respectively. The BMD_{05} and $BMDL_{05}$ values for the selected model were 0.702 and 0.122 mg/kg-day, respectively; the BMD_{01} and $BMDL_{01}$ values for the selected model were 0.137 and 0.00906 mg/kg-day, respectively.

[b]The Gamma model had a power parameter estimate of 1 (boundary of parameter space). The Multistage 2°, 3°, and 4° models had b2, b3, and b4 coefficients of 0 (boundary of parameter space). The models in this row are equivalent to the Quantal-Linear model.

[c]The Weibull model may appear equivalent to the Quantal-Linear model; however, differences exist in digits not displayed in the table.

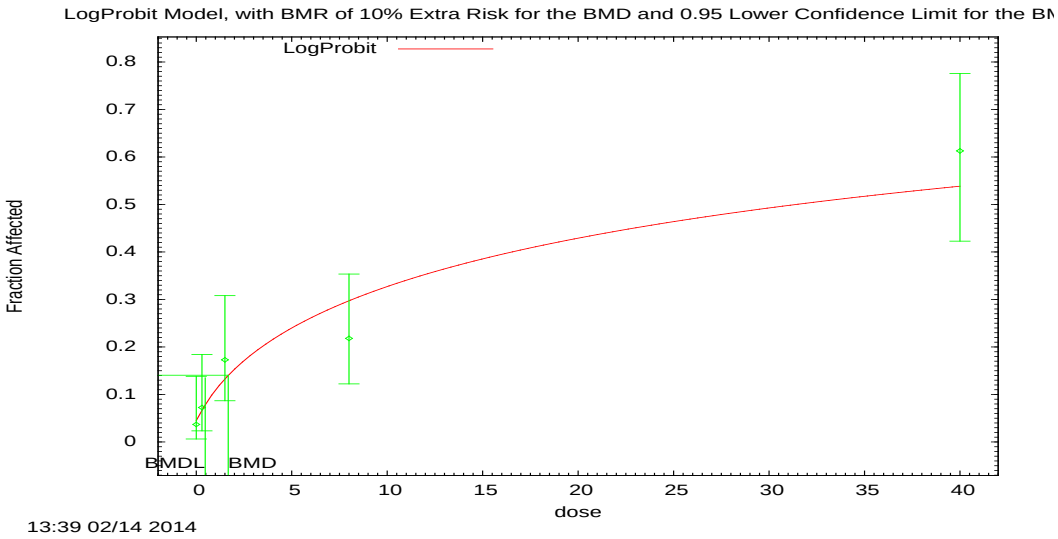


Figure D-7. Plot of incidence rate by dose, with fitted curve for selected model, for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983).

Probit Model (Version: 3.3; Date: 2/28/2013)

The form of the probability function is: P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)), where CumNorm(.) is the cumulative normal distribution function

Slope parameter is not restricted

Benchmark Dose Computation

BMR = 10% ER

BMD = 1.67454

BMDL at the 95% confidence level = 0.468688

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0452202	0.037037
Intercept	−1.4970E+00	−1.3564E+00
Slope	0.417872	0.36341

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−96.3905	5			
Fitted model	−98.0147	3	3.24837	2	0.1971
Reduced model	−118.737	1	44.6933	4	<0.0001

AIC: = 202.029

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0452	2.442	2	54	−0.289
0.3	0.0669	3.682	4	55	0.172
1.5	0.1332	6.927	9	52	0.846
8	0.2982	16.402	12	55	−1.298
40	0.5396	16.726	19	31	0.819

Chi^2 = 3.18 d.f. = 2 *p*-value = 0.2035

D.1.3. Mortality: Dose-Response Analysis and BMD Modeling Documentation

This appendix also presents a quantitative dose-response analysis of mortality incidence from studies identified in Section 2.1.6 (see Table D-10).

Table D-10. Mortality data selected for dose-response modeling for RDX

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
Lish et al. (1984) (mortality at 11 wks)	Male B6C3F$_1$ mouse	0 mg/kg-d 1.5 7 35 175/100	1 / 85 (1%) 0 / 85 (0%) 0 / 85 (0%) 0 / 85 (0%) 30 / 85 (35%)
Lish et al. (1984) (mortality at 11 wks)	Female B6C3F$_1$ mouse	0 mg/kg-d 1.5 7 35 175/100	0 / 85 (1%) 0 / 85 (0%) 0 / 85 (0%) 0 / 85 (0%) 36 / 85 (42%)
Levine et al. (1981b)[a]	Female F344 rat	0 mg/kg-d 10 30 100 300 600	0 / 30 (0%) 1 / 10 (10%) 0 / 10 (0%) 5 / 10 (50%) 10 / 10 (100%) 10 / 10 (100%)

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
	Male F344 rat	0 mg/kg-d 10 30 100 300 600	0 / 30 (0%) 0 / 10 (0%) 0 / 10 (0%) 8 / 10 (80%) 10 / 10 (100%) 10 / 10 (100%)
	Male and female F344 rat, combined	0 mg/kg-d 10 30 100 300 600	0 / 60 (0%) 1 / 20 (5%) 0 / 20 (0%) 13 / 20 (65%) 20 / 20 (100%) 20 / 20 (100%)
von Oettingen et al. (1949)	Rats, sex/strain not specified	0 mg/kg-d 15 25 50	0 / 20 (0%) 0 / 19 (0%)[b] 8 / 20 (40%) 8 / 20 (40%)
Cholakis et al. (1980) (2-generation study)	Female CD rat	0 mg/kg-d 5 16 50	0 / 22 (0%) 0 / 22 (0%) 0 / 22 (0%) 6 / 22 (27%)
Levine et al. (1983) (mortality at 13 wks)	Male F344 rat	0 mg/kg-d 0.3 1.5 8 40	0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%)
	Female F344 rat	0 mg/kg-d 0.3 1.5 8 40	0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%) 0 / 75 (0%)
Cholakis et al. (1980) (13-wk study)	Male F344 rat	0 mg/kg-d 10 14 20 28 40	0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%)
	Female F344 rat	0 mg/kg-d 10 14 20 28 40	0 / 9 (0%)[c] 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%) 0 / 10 (0%)

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
Crouse et al. (2006)	Female F344 rat	0 mg/kg-d 4 8 10 12 15	0 / 10 (0%) 0 / 10 (0%) 1 / 10 (20%) 2 / 10 (20%) 5 / 10 (50%) 4 / 10 (40%)
	Male F344 rat	0 mg/kg-d 4 8 10 12 15	0 / 10 (0%) 0 / 10 (0%) 1 / 10 (10%) 3 / 10 (30%) 2 / 10 (20%) 3 / 10 (30%)
	Male and female F344 rat, combined	0 mg/kg-d 4 8 10 12 15	0 / 20 (0%) 0 / 20 (0%) 2 / 20 (10%) 5 / 20 (25%) 7 / 20 (35%) 7 / 20 (35%)
Cholakis et al. (1980) (gestational exposure)	Female F344 rats (gestational exposure)	0 mg/kg-d 0.2 2 20	0 / 24 (0%) 0 / 24 (0%) 0 / 24 (0%) 5 / 24 (21%)
Angerhofer et al. (1986)	Female SD rate (gestational exposure)	0 mg/kg-d 2 6 20	0 / 39 (0%) 1 / 40 (3%) 1 / 40 (3%) 16 / 51 (31%)
Cholakis et al. (1980)	Female New Zealand white rabbit (gestational exposure)	0 mg/kg-d 0.2 2 20	0 / 11 (0%) 0 / 11 (0%) 0 / 11 (0%) 0 / 12 (0%)

[a]For Levine et al. (1981a) and Crouse et al. (2006), the incidence rates across doses were determined to be not statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for each of these endpoints prior to modeling.
[b]For von Oettingen et al. (1949), one mortality was reported in the 15 mg/kg-day dose group. However, this mortality was most likely not related to RDX, so the animal that died was excluded.
[c]For Cholakis et al. (1980), one accidental death was reported in the 0 mg/kg-day dose group. The animal that died was excluded.

Tables D-11 to D-14 present the BMD modeling results for incidence of mortality from Crouse et al. (2006), von Oettingen et al. (1949), Levine et al. (1983), and Angerhofer et al. (1986). The following datasets were not modeled because each had either no response or a positive response only in the highest dose group: 11-week mortality from Lish et al. (1984), both male (one death in control group) and female; 13-week mortality data from Levine et al. (1983), both male

and female; mortality in female CD rats and male and female F344 rats from Cholakis et al. (1980); and mortality in female F344 rats during gestational exposure from Cholakis et al. (1980).

Table D-11. BMD modeling results for combined mortality in male and female F344 rats exposed to RDX by diet for 13 weeks (Levine et al., 1981b); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.401	41.100	49.8	12.7	The quantal-linear model was excluded because the fit has a residual below -2 at 4 mg/kg-d. The multistage 4° model was selected as the representative multistage model based on lowest AIC. From among the multistage 4° and non-multistage models, the multistage 4° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.346	41.429	18.3	8.25	
LogLogistic	0.257	43.098	73.2	16.9	
Probit	0.328	41.727	15.0	6.82	
LogProbit	0.257	43.098	58.6	19.5	
Weibull	0.257	43.101	56.6[b]	6.51[b]	
Multistage 2°	0.424	42.942	7.72	2.01	
Quantal-Linear	0.139	50.257	1.12	0.818	
Multistage 3°	0.503	41.520	7.71	2.08	
Multistage 4°	**0.535**	**40.935**	**7.85**	**2.15**	
Multistage 5°	0.371	42.928	7.86	2.15	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 10, 30, 100, 300, and 600 mg/kg-day were 0.00, 1.48, −0.97, 0.05, 0.00, and 0.00, respectively. The BMD_{10} and $BMDL_{10}$ estimates for the selected model were 47.2 and 22.2 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ estimates for the selected model were 32.4 and 11.0 mg/kg-day, respectively.

[b]The parameter convergence parameter was increased to 2×10^{-8} to obtain convergence.

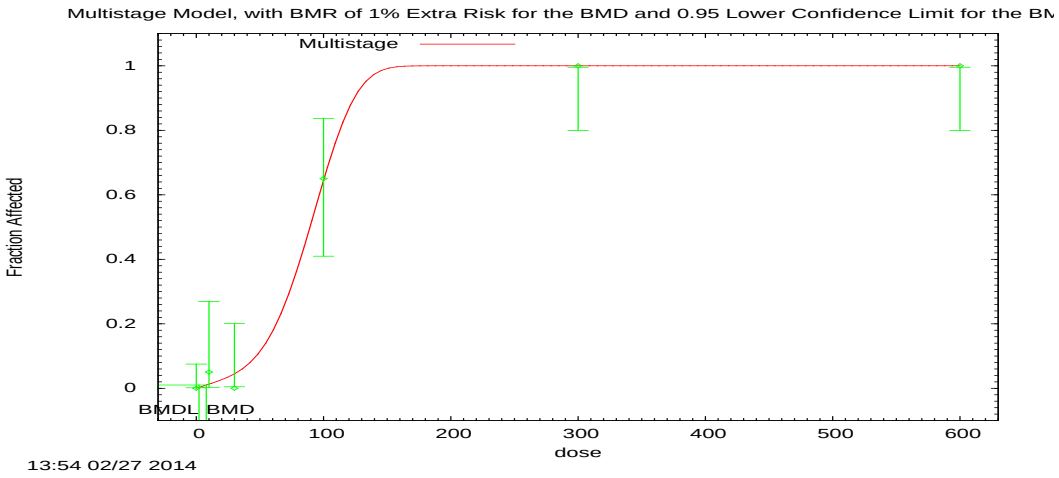


Figure D-8. Plot of incidence rate by dose, with the fitted curve of the multistage 2° model, for combined mortality in male and female F344 rats exposed to RDX by diet for 13 weeks (Levine et al., 1981b); BMR = 1% ER.

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% Extra risk

BMD = 7.85287

BMDL at the 95% confidence level = 2.15059

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0.00127544	1.9710E+17
Beta(2)	0	0
Beta(3)	0	0
Beta(4)	9.0721E−09	0

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−16.9192	6			
Fitted model	−18.4677	2	3.09685	4	0.5418
Reduced model	−102.298	1	170.758	5	<0.0001

AIC: = 40.9353

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	60	0
10	0.0128	0.255	1	20	1.484
30	0.0446	0.892	0	20	−0.966
100	0.6447	12.894	13	20	0.05
300	1	20	20	20	0
600	1	20	20	20	0

Chi^2 = 3.14 d.f. = 4 *p*-value = 0.5352

Table D-12. BMD modeling results for mortality (number found dead) in rats exposed to RDX in the diet for 13 weeks (von Oettingen et al., 1949)

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.0341	66.088	3.14	0.648	All of the models besides dichotomous-Hill had goodness-of-fit *p*-values <0.10 and thus did not provide an adequate fit to the data. For the dichotomous-Hill model, the slope parameter acheived the BMDS internal upper bound (18), so the results from this model were not reliable. No model was selected.
Dichotomous-Hill	0.984	57.888	17.2	10.9	
Logistic	0.0044	70.074	3.40	2.08	
LogLogistic	0.0397	65.853	3.39	0.529	
Probit	0.0056	69.283	3.28	1.94	
LogProbit	0.0426	65.464	5.67	0.409	
Weibull	0.0349	66.233	2.30	0.641	
Multistage 3°[a]	0.0351	66.517	1.22	0.628	
Multistage 2°[b]	0.0351	66.517	1.22	0.628	
Quantal-Linear	0.0995	64.639	0.919	0.623	

[a]The Multistage 3° model may appear equivalent to the Multistage 2° model; however, differences exist in digits not displayed in the table.
[b]The Multistage 2° model may appear equivalent to the Multistage 3° model; however, differences exist in digits not displayed in the table.

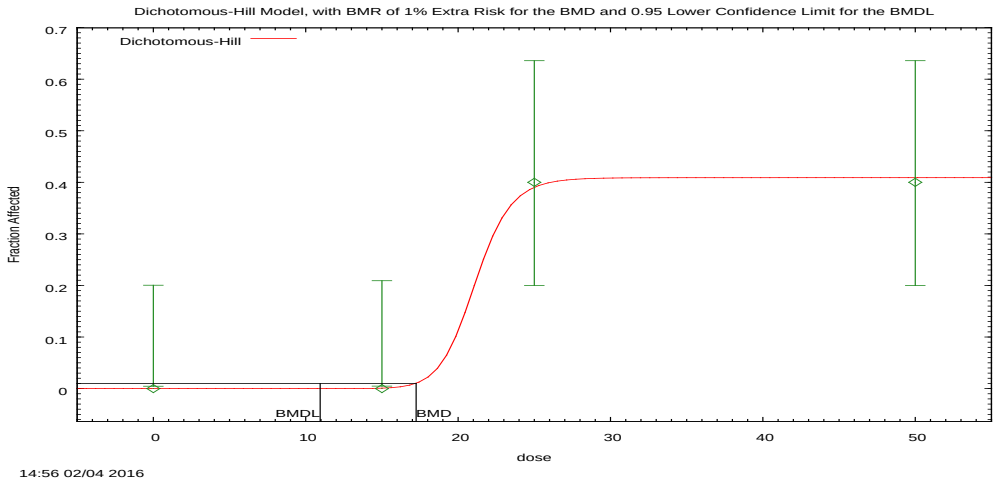


Figure D-9. Plot of incidence rate by dose with fitted curve for Dichotomous-Hill model for Model predictions for mortality (number found dead) in rats exposed to RDX in the diet for 13 weeks (von Oettingen et al., 1949); dose shown in mg/kg-day.

Table D-13. BMD modeling results for combined mortality (number found dead) in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR= 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.794	93.263	3.46	0.840	The multistage 2° model was selected as the representative multistage model based on lowest AIC. From among the multistage 2° and non-multistage models, the multistage 2° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.474	95.709	2.11	1.11	
LogLogistic	0.794	93.332	3.17	0.872	
Probit	0.574	94.797	2.40	1.07	
LogProbit	0.854	92.832	3.96	1.48	
Weibull	0.743	93.698	2.76	0.641	
Multistage 2°	**0.858**	**91.926**	**2.11**	**0.463**	
Quantal-Linear	0.535	95.345	0.405	0.288	
Multistage 5°[b]	0.731	93.851	2.42	0.433	
Multistage 4°[c]	0.731	93.851	2.42	0.433	
Multistage 3°	0.731	93.851	2.42	0.439	
Dichotomous-Hill	0.998	93.343	5.96	1.95	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, −0.86, −0.46, 0.53, 0.72, and 0.45, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 6.82 and 4.41 mg/kg-d, respectively.

[b]The Multistage 5° model may appear equivalent to the Multistage 4° model; however, differences exist in digits not displayed in the table.

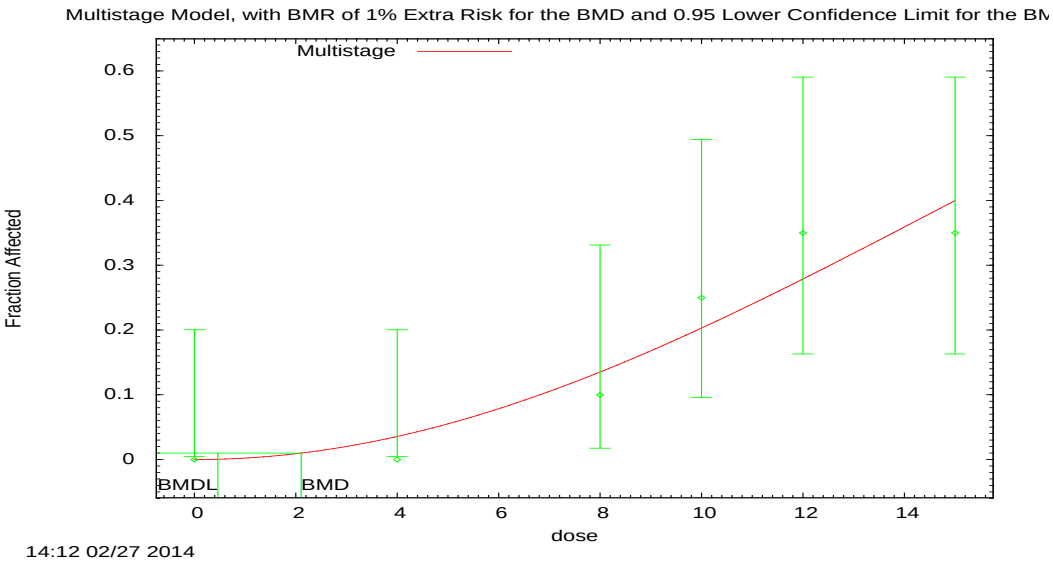


Figure D-10. Plot of incidence rate by dose, with the fitted curve of the multistage 2° model, for mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% ER; dose shown in mg/kg-day.

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation.

BMR = 1% Extra risk

BMD = 2.10625

BMDL at the 95% confidence level = 0.462994

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.0134587
Beta(2)	0.00226548	0.00141278

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−43.6462	6			
Fitted model	−44.963	1	2.63354	5	0.7563
Reduced model	−55.6472	1	24.0019	5	0.0002169

AIC: = 91.926

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.0356	0.712	0	20	−0.859
8	0.135	2.699	2	20	−0.458
10	0.2027	4.054	5	20	0.526
12	0.2784	5.567	7	20	0.715
15	0.3993	7.987	7	20	−0.451

Chi^2 = 1.94 d.f. = 5 *p*-value = 0.8576

Table D-14. Model predictions for mortality in female Sprague-Dawley rats exposed to RDX by gavage on gestation days 6–15 (Angerhofer et al., 1986); BMR = 1% ER

Model[a]	Goodness of fit		BMD_{1Pct} (mg/kg-d)	$BMDL_{1Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.314	89.496	5.15	0.538	The multistage 3° model was selected as the representative multistage model based on lowest AIC. From among the multistage 3° and non-multistage models, the multistage 3° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.667	87.213	3.88	2.16	
LogLogistic	0.312	89.473	4.88	0.560	
Probit	0.643	87.196	3.37	1.87	
LogProbit	0.319	89.522	5.58	0.885	
Weibull	0.309	89.458	4.62	0.541	
Quantal-Linear	0.450	87.502	0.652	0.452	
Multistage 3°	**0.655**	**86.906**	**1.68**	**0.588**	
Multistage 2°	0.554	87.291	1.78	0.555	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 2, 6, and 20 mg/kg-day were 0.00, 0.76, −0.52, and 0.04, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 10.9 and 6.09 mg/kg-day, respectively; the BMD_{05} and $BMDL_{05}$ estimates for the selected model were 5.23 and 7.29 mg/kg-day, respectively.

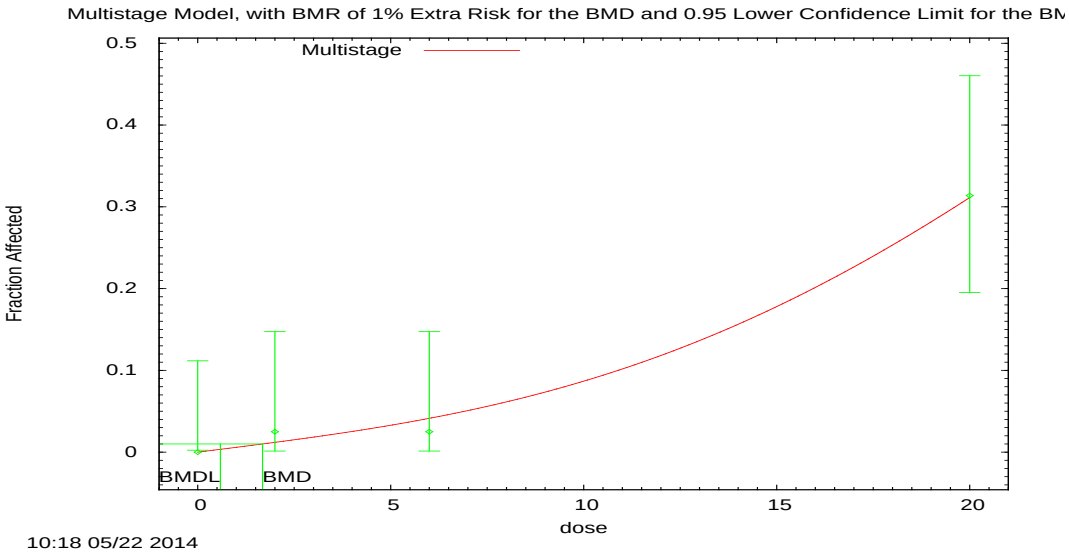


Figure D-11. Plot of incidence rate by dose, with the fitted curve of the multistage 3° model, for mortality in female Sprague-Dawley rats exposed to RDX by gavage on gestation days 6–15 (Angerhofer et al., 1986); BMR = 1% ER.

Multistage Model (Version: 3.3; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

Benchmark Dose Computation

BMR = 1% Extra risk

BMD = 1.68097

BMDL at the 95% confidence level = 0.587568

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0.00807857
Beta(1)	0.00588873	0.00216407
Beta(2)	0	0
Beta(3)	0.0000319123	0.0000406218

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−41.0771	4			
Fitted model	−41.4531	2	0.752152	2	0.6866
Reduced model	−57.4292	1	32.7043	3	<0.0001

AIC: = 86.9063

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	39	0
2	0.012	0.478	1	40	0.759
6	0.0413	1.654	1	40	−0.519
20	0.3114	15.881	16	51	0.036

Chi^2 = 0.85 d.f. = 2 *p*-value = 0.6549

D.2. BENCHMARK DOSE MODELING SUMMARY FOR CANCER ENDPOINTS

The cancer endpoints in the mouse that were selected for dose-response modeling are presented in Table D-15. For each endpoint, the doses and tumor incidence data used for the modeling are presented.

Table D-15. Cancer endpoints selected for dose-response modeling for RDX

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total
Hepatocellular adenomas or carcinomas Parker et al. (2006)	Female B6C3F$_1$ mouse	0 1.5 7 35 107	1/67 (1%)[a] 4/62 (6%) 5/63 (8%) 10 /64 (16%) 4/31 (13%)
Alveolar/bronchiolar adenomas or carcinomas Lish et al. (1984)	Female B6C3F$_1$ mouse	0 1.5 7 35 107	7/65 (11%) 3/62 (5%) 8/64 (13%) 12/64 (19%) 7/31 (23%)

[a]For female mouse hepatocellular tumors from Lish et al. (1984), tumor incidence and totals are those reported in the Pathology Working Group (PWG) reevaluation (Parker et al., 2006).

D.2.1. Evaluation of Model Fit and Model Selection for Mouse Tumor Data

First, to determine whether a time-to-tumor analysis was warranted, the survival curves were compared across dose groups for female mice in Lish et al. (1984) in the study to determine whether time of death should be incorporated in the dose-response analysis of tumors. A log-rank test on the Kaplan-Meier survival curves per dose was used to do the comparison, excluding animals that died prior to week 11 when the dose was reduced in the high-dose group to 100 mg/kg-day. The test yielded a nonsignificant result ($p = 0.51$), so a time-to-tumor analysis was not necessary for this study.

Therefore, non-time-dependent dose-response analyses were conducted using standard BMDS models. For each tumor type, BMDS multistage-cancer models[6] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value <0.05[7] indicates lack of fit). Other factors were used to assess model fit, including scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR. The BMDL estimate and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close" (i.e., differed by threefold or less), then the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, then the lowest BMDL was selected as the POD.

After selecting models for the two endpoints, the results were combined using MS-COMBO in BMDS. This procedure analyzes the incidence of a tumor (adenoma or carcinoma) defined as present if either the hepatocellular or alveolar/bronchiolar tumor (or both) was present, and not present otherwise. The two endpoints were assumed to be independent.

D.2.2. Modeling Results for Mouse Tumor Data

The BMD modeling results for mouse tumor data sets are provided in Tables D-16 to D-20 (and Figures D-12 to D-16).

[6]The coefficients of the multistage-cancer models were restricted to be nonnegative (beta values ≥0).
[7]A significance level of 0.05 from U.S. EPA (2012b) is used for selecting cancer models because the model family (multistage) is selected a priori.

Mouse Tumor Data—BMD Modeling Results

Table D-16. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b] **Multistage 2°** **Multistage 3°** **Multistage 4°**	**0.417**	**218.68**	**52.8**	**27.7**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were 0.40, −1.27, 0.50, 0.73, and −0.52, respectively.

[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

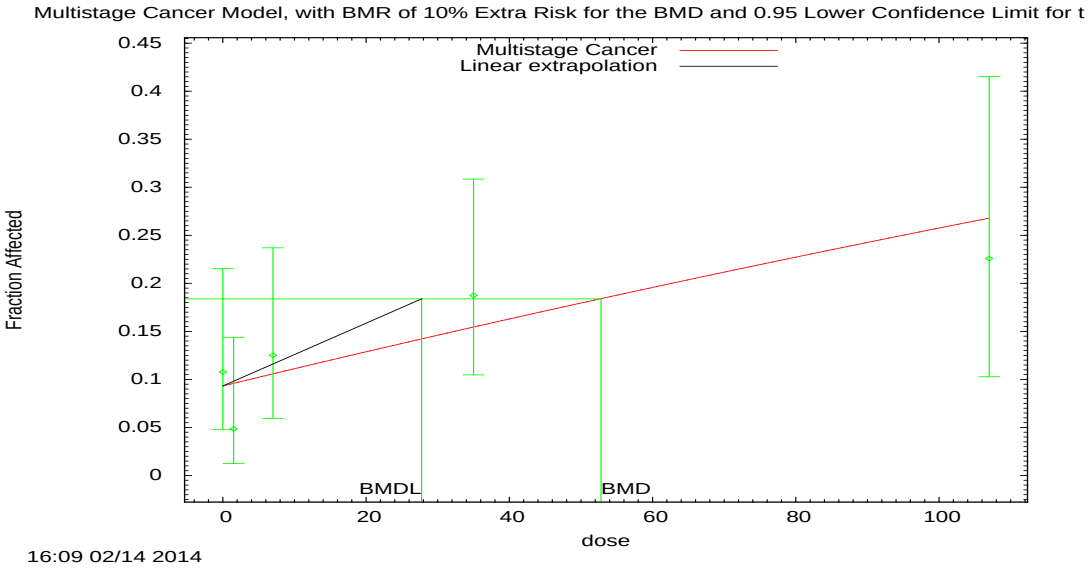


Figure D-12. Plot of incidence rate by dose, with the fitted curve for the selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984).

Multistage Cancer Model (Version: 1.10; Date: 02/28/2013)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 10% ER

BMD = 52.8078

BMDL at the 95% confidence level = 27.748

Benchmark dose upper bound (BMDU) at the 95% confidence level = 194.806

Taken together, (27.748, 194.806) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00360387

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.093168	0.0998927
Beta(1)	0.00199517	0.00155773

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−105.777	5			
Fitted model	−107.341	2	3.12764	3	0.3724
Reduced model	−110.164	1	8.77367	4	0.06701

AIC: = 218.682

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0932	6.056	7	65	0.403
1.5	0.0959	5.944	3	62	−1.27
7	0.1057	6.768	8	64	0.501
35	0.1543	9.877	12	64	0.734
107	0.2675	8.292	7	31	−0.524

Chi^2 = 2.84 d.f. = 3 *p*-value = 0.4168

Table D-17. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 5% ER

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b] **Multistage 2°** **Multistage 3°** **Multistage 4°**	**0.417**	**218.68**	**25.7**	**13.5**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were 0.40, −0.40, −1.27, 0.50, 0.73, and −0.52, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 52.8 and 27.7 mg/kg-day, respectively.

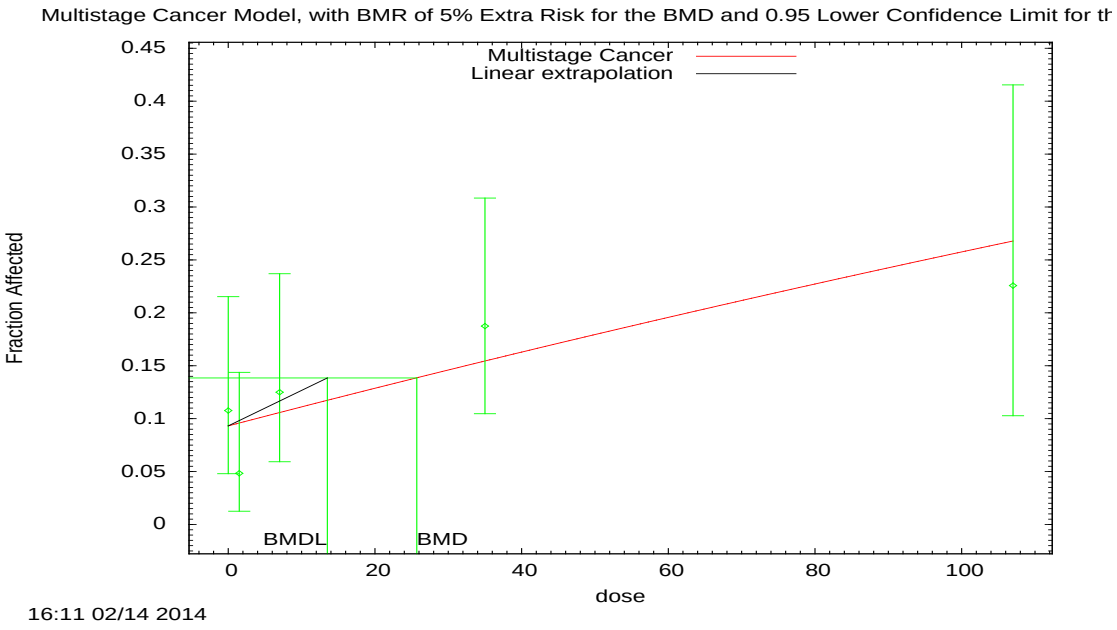


Figure D-13. Plot of incidence rate by dose, with fitted curve for selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984).

Multistage Cancer Model (Version: 1.10; Date: 02/28/2013)
The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation
BMR = 5% ER
BMD = 25.7088
BMDL at the 95% confidence level = 13.5087
BMDU at the 95% confidence level = 94.8384
Taken together, (13.5087, 94.8384) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.00370131

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.093168	0.0998927
Beta(1)	0.00199517	0.00155773

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−105.777	5			
Fitted model	−107.341	2	3.12764	3	0.3724
Reduced model	−110.164	1	8.77367	4	0.06701

AIC: = 218.682

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0932	6.056	7	65	0.403
1.5	0.0959	5.944	3	62	−1.27
7	0.1057	6.768	8	64	0.501
35	0.1543	9.877	12	64	0.734
107	0.2675	8.292	7	31	−0.524

Chi^2 = 2.84 d.f. = 3 *p*-value = 0.4168

Table D-18. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b]	**0.160**	**164.06**	**64.2**	**32.6**	All of the models reduced to the Multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					
Multistage 4°					

[a]Selected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were −1.37, 0.35, 0.54, 1.34, and −1.05, respectively.

[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

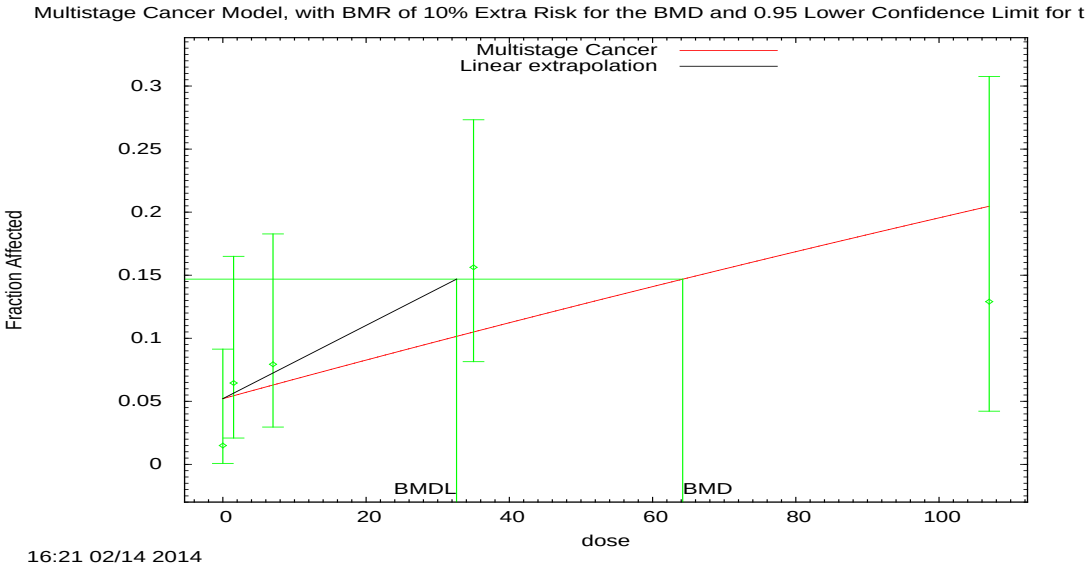


Figure D-14. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Parker et al., 2006).

Multistage Cancer Model (Version: 1.10; Date: 02/28/2013)
The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation
BMR = 10% ER
BMD = 64.203
BMDL at the 95% confidence level = 32.6282
BMDU at the 95% confidence level = 281.385
Taken together, (32.6282, 281.385) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.00306483

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0520755	0.0658334
Beta(1)	0.00164105	0.000876864

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−77.1516	5			
Fitted model	−80.0315	2	5.75967	3	0.1239
Reduced model	−82.5216	1	10.74	4	0.02965

AIC: = 164.063

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0521	3.489	1	67	−1.369
1.5	0.0544	3.373	4	62	0.351
7	0.0629	3.963	5	63	0.538
35	0.105	6.719	10	64	1.338
107	0.2047	6.347	4	31	−1.045

Chi^2 = 5.17 d.f. = 3 *p*-value = 0.16

Table D-19. Model predictions for B6C3F$_1$ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 5% ER

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b]	**0.160**	**164.06**	**31.3**	**15.9**	All of the models reduced to the Multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					
Multistage 4°					

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were −1.37, 0.35, 0.54, 1.34, and −1.05, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 64.2 and 32.6 mg/kg-day, respectively.

[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

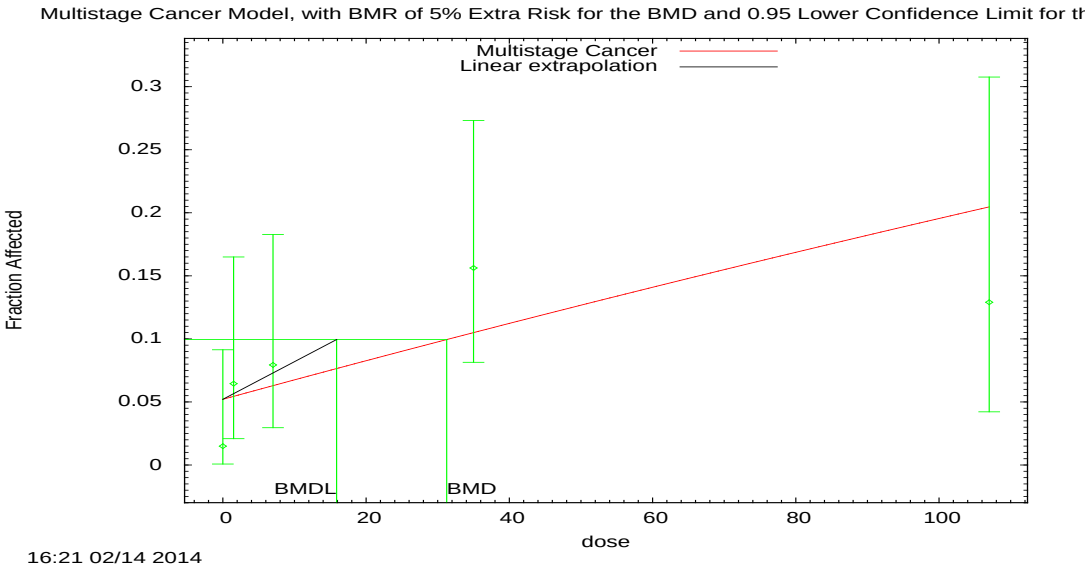


Figure D-15. Plot of incidence rate by dose, with fitted curve for selected model, for B6C3F$_1$ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006).

Multistage Cancer Model (Version: 1.10; Date: 02/28/2013)
The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation
BMR = 5% ER
BMD = 31.2563
BMDL at the 95% confidence level = 15.8846
BMDU at the 95% confidence level = 136.989
Taken together, (15.8846, 136.989) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.0031477

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0520755	0.0658334
Beta(1)	0.00164105	0.000876864

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−77.1516	5			
Fitted model	−80.0315	2	5.75967	3	0.1239
Reduced model	−82.5216	1	10.74	4	0.02965

AIC: = 164.063

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0521	3.489	1	67	−1.369
1.5	0.0544	3.373	4	62	0.351
7	0.0629	3.963	5	63	0.538
35	0.105	6.719	10	64	1.338
107	0.2047	6.347	4	31	−1.045

Chi^2 = 5.17 d.f. = 3 *p*-value = 0.16

Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or carcinoma in B6C3F$_1$ female mice exposed to RDX by diet for 24 months; BMR = 10% ER

BMD = 29.0 mg/kg-day; BMDL = 17.7 mg/kg-day

MSCOMBO results

BMR of 10% Extra Risk

```
**** Start of combined BMD and BMDL Calculations.****

 Combined Log-Likelihood           -187.3723596892213

 Combined Log-likelihood Constant       166.01737626058841


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    28.9753

      BMDL =    17.6574

Multistage Cancer Slope Factor =  0.00566334
```

Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or carcinoma in B6C3F$_1$ female mice exposed to RDX by diet for 24 months; BMR = 5% ER

BMD = 29.0 mg/kg-day; BMDL = 17.7 mg/kg-day

MSCOMBO results

BMR of 5% Extra Risk

```
**** Start of combined BMD and BMDL Calculations.****

 Combined Log-Likelihood           -187.3723596892213

 Combined Log-likelihood Constant       166.01737626058841


  Benchmark Dose Computation

Specified effect =      0.05

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    14.1062

      BMDL =    8.59627

Multistage Cancer Slope Factor =  0.00581647
```

Table D-20. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months, using incidence frequencies from Parker et al. (2006) and sample sizes from Lish et al. (1984); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b]	**0.171**	**163.98**	**64.8**	**32.8**	All of the models reduced to the Multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					
Multistage 4°					

[a]Selected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were −1.34, 0.34, 0.49, 1.34, and −1.03, respectively.

[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

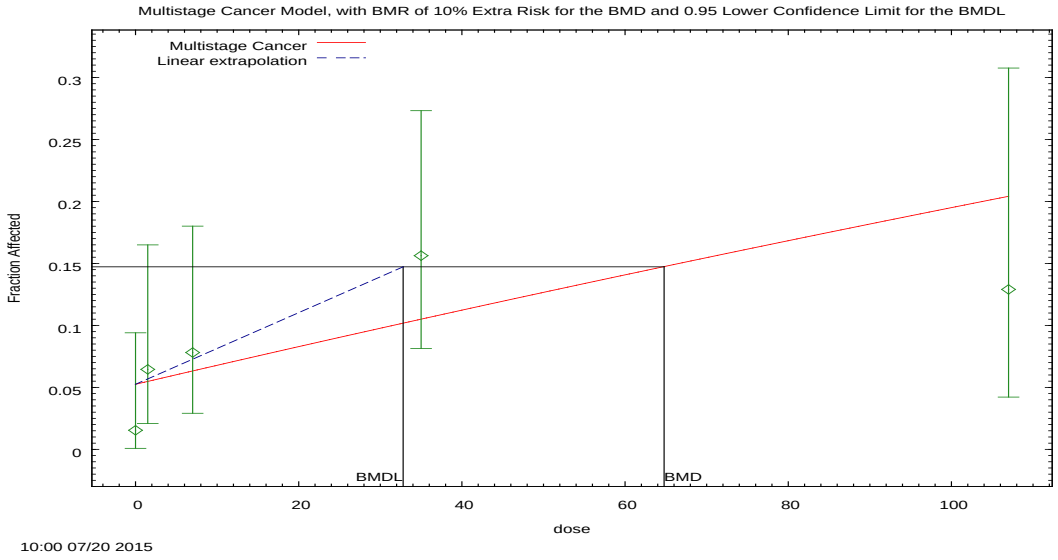


Figure D-16. Plot of incidence rate by dose with fitted curve for Multistage-Cancer 1° model for model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months, using incidence frequencies from Parker et al. (2006) and sample sizes from Lish et al. (1984).

Multistage Model (Version: 3.4; Date: 05/02/2014)
The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]
The parameter betas are restricted to be positive

Benchmark Dose Computation
BMR = 10% Extra risk
BMD = 64.7853
BMDL at the 95% confidence level = 32.7981
BMDU at the 95% confidence level = 291.495
Taken together, (32.7981, 291.495) is a 90% two-sided confidence interval for the BMD
Multistage Cancer Slope Factor = 0.00304896

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0525105	0.0656105
Beta(1)	0.0016263	0.000878945

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−77.2	5			
Fitted model	−79.99	2	5.57217	3	0.13
Reduced model	−82.43	1	10.462	4	0.03

AIC: = 163.978

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0525	3.413	1	65	−1.34
1.5	0.0548	3.399	4	62	0.34
7	0.0632	4.047	5	64	0.49
35	0.1049	6.716	10	64	1.34
107	0.2038	6.319	4	31	−1.03

Chi^2 = 5.02 d.f. = 3 *p*-value = 0.1706

Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or carcinoma in B6C3F$_1$ female mice exposed to RDX by diet for 24 months; for hepatocellular adenoma or carcinoma, the incidence frequencies from Parker et al. (2006) and the sample sizes from Lish et al. (1984) were used; BMR = 10% ER

BMD = 29.0 mg/kg-day; BMDL = 17.7 mg/kg-day

MSCOMBO results

BMR of 10% Extra Risk

```
**** Start of combined BMD and BMDL Calculations.****

  Combined Log-Likelihood                    -187.33008597565913

  Combined Log-likelihood Constant              166.068416550547


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =        29.0933

            BMDL =        17.7048

Multistage Cancer Slope Factor =    0.00564818
```

D.2.3. Dose-response Analysis and BMD Modeling Documentation for Other Tumor Data Sets

This appendix also presents a quantitative dose-response analysis of incidence of liver carcinomas in male F344 rats (Levine et al., 1983) and incidence of lung carcinomas in male B3C6F$_1$ mice (Table D-21). The resulting candidate oral slope factors (OSFs) are presented for comparison with OSF estimates provided in Section 2.3.3 of the Toxicological Review.

Table D-21. Liver carcinoma data from Levine et al. (1983)

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total
Alveolar/bronchiolar carcinomas Lish et al. (1984)	Male B6C3F$_1$ mouse	0 1.5 7 35 107	3/63 (5%) 6/60 (10%) 3/62 (5%) 7/59 (12%) 5/27 (19%)
Hepatocellular carcinomas Levine et al. (1983)	Male F344 rat	0 0.3 1.5 8 40	1/55 (2%) 0/55 (0%) 0/52 (0%) 2/55 (4%) 2/31[a] (6%)

[a]The denominators listed in the table represent the number of animals that were alive 1 year after dosing began.

For male mice in Lish et al. (1984), a log-rank test on the Kaplan-Meier survival curves, stratified by dose, yielded a nonsignificant result (p-value ≥0.10), indicating that the survival curves were similar across dose groups. Therefore, a time-to-tumor analysis was not necessary for hepatocellular carcinomas in Lish et al. (1984). A non-time-dependent dose-response analysis was conducted using BMDS multistage-cancer models, and the model selection procedures described in Section D.2.1 were used to select the appropriate models. Subsequently, the administered dose was converted to a human equivalent dose (HED) on the basis of (body weight)$^{3/4}$ (U.S. EPA, 1992), as described in Section 2.3.2. The POD estimate for male mouse carcinomas and OSF calculated from this POD are provided in Table D-22; detailed BMD modeling results are provided in Table D-23 (and Figure D-17).

Table D-22. Model predictions and oral slope factor for alveolar/bronchiolar carcinomas in male B6C3F$_1$ mice exposed to RDX by diet for 2 years (Lish et al., 1984)

Tumor type	Selected model	BMR	BMD, mg/kg-d	BMDL, mg/kg-d	POD = $BMDL_{10\text{-}HED}$[a] mg/kg-d	Candidate OSF[b] $(mg/kg\text{-}d)^{-1}$
Alveolar/bronchiolar carcinomas	Multistage 1°	10% ER	76.1	36.2	5.41	0.018

[a]Based on allometric scaling of administered RDX dose; $BMDL_{10\text{-}HED} = BMDL_{10} \times (BW_a^{1/4}/BW_h^{1/4})$, $BW_a = 0.035$ kg, and $BW_h = 70$ kg.
[b]Slope factor = $BMR/BMDL_{10\text{-}HED}$, where BMR = 0.10 (10% ER).

Table D-23. Summary of BMD modeling results for model predictions for alveolar/bronchiolar carcinoma in male B6C3F$_1$ mice exposed to RDX by diet for 2 years (Lish et al., 1984); BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b]	**0.561**	**162.00**	**76.1**	**36.2**	All of the models reduced to the multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					
Multistage 4°					

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were −0.52, 1.08, −0.74, 0.27, and −0.1, respectively.
[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

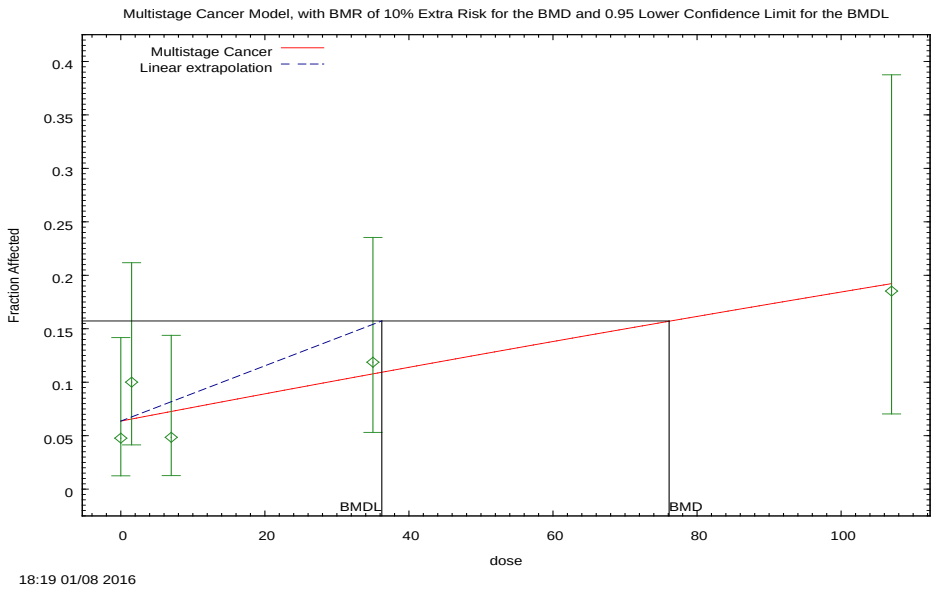


Figure D-17. Plot of incidence rate by dose with fitted curve for Multistage-Cancer 1° model for Model predictions for alveolar/bronchiolar carcinoma in male B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984).

Multistage Model (Version: 3.4; Date: 05/02/2014)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 10% Extra risk

BMD = 76.1197

BMDL at the 95% confidence level = 36.2316

BMDU at the 95% confidence level = 27443100

Taken together, (36.2316, 27443100) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00276003

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0635642	0.0652915
Beta(1)	0.00138414	0.00131052

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−78	5			
Fitted model	−79	2	1.99294	3	0.57
Reduced model	−81.08	1	6.15622	4	0.19

AIC: = 162.001

Goodness-of-Fit Table

Dose	Est. Prob.	Expected	Observed	Size	Scaled residuals
0	0.0636	4.005	3	63	−0.52
1.5	0.0655	3.93	6	60	1.08
7	0.0726	4.501	3	62	−0.74
35	0.1078	6.363	7	59	0.27
107	0.1925	5.197	5	27	−0.1

Chi^2 = 2.06 d.f. = 3 *p*-value = 0.561

For male rats in Levine et al. (1983), the high-dose group had a markedly lower survival curve than the other dose groups, indicating a substantial number of early deaths in the high-dose group. A log-rank test on the Kaplan-Meier survival curves, stratified by dose, yielded a significant result (*p*-value <0.001), in which case a time-to-tumor analysis is generally preferred. However, such an analysis was not possible because the data were insufficient to allow this analysis. Although tumor incidence was listed for each animal in the source article, the pathology report used a different animal numbering system than the experimental report where the times of death were listed, and the relationship between the two systems was not documented. This prohibited the matching of the times of death and the tumor incidence of the animals, thus prohibiting the use of a time-to-tumor analysis.

Therefore, a non-time-dependent dose-response analysis was conducted using BMDS multistage-cancer models. The model selection procedures described in Section D.2.1 were used to select the appropriate models. To account for the difference in the survival curves across the groups for rats, the number of animals alive at 12 months was used as the denominator in the analysis (denominators listed in Table D-21). Because the maximum liver tumor response in the male rat was 6.4%, a BMR of 5% was used to model male rat liver tumor data in order to obtain a BMD and BMDL in the range of the experimental data, as recommended in Section 3.2 of *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

To estimate the HED at the BMDL, HEDs based on both administered dose scaled by $BW^{3/4}$ and physiologically based pharmacokinetic (PBPK) modeling were considered. Confidence in the revised rat PBPK model is relatively high (see Appendix C, Section C.1.5); however, the choice of an

internal dose is not straightforward. First, evidence regarding the involvement of metabolites has been discussed in the literature only in the context of the mouse, and the rate of metabolism (allometrically adjusted) appears to be qualitatively slower for the rat. Second, metabolism in the model represents the total of all pathways, whereas it is only the minor N-nitroso metabolic route, and not the oxidative route that has been proposed as a factor in RDX-induced mouse carcinogenicity. Third, while blood concentration of RDX as an internal dose would be more proximally relevant to the tissue than administered dose, there are no data to indicate that the parent RDX is directly related to its carcinogenicity. Therefore, given the uncertainties, HEDs based on both administered dose scaled by $BW^{3/4}$ and area under the curve (AUC) of RDX arterial blood concentration (calculated using the PBPK model) are presented. Extrapolation based on the internal dose of the parent compound is accomplished by assuming toxicological equivalence when dose is expressed in terms of the AUC of the RDX blood concentration.

The POD estimates for rat liver carcinomas and the OSFs calculated from these PODs are provided in Table D-24; detailed BMD modeling results are provided in Table D-25 (and Figure D-18). Results based on two dose-metrics are presented: administered dose of RDX scaled by $BW^{3/4}$ (when dose is expressed in terms of mg/kg-day, this entails scaling by $BW^{-1/4}$) and AUC of RDX arterial blood concentration (using PBPK modeling).

Table D-24. Model predictions and oral slope factor for hepatocellular carcinomas in male F344 rats administered RDX in the diet for 2 years (Levine et al., 1983)

Tumor type	Selected model	BMR	BMD, mg/kg-d	BMDL, mg/kg-d	POD = $BMDL_{05\text{-}HED}$, mg/kg-d	Candidate OSF[a] $(mg/kg\text{-}d)^{-1}$
Hepatocellular carcinomas	Multistage 1°	5% ER	28.5	11.8	2.88[b], 5.75[c]	0.017[b], 0.009[c]

[a]Slope factor = $BMR/BMDL_{05\text{-}HED}$, where BMR = 0.05 (5% ER).
[b]Based on allometric scaling of administered RDX dose; $BMDL_{05\text{-}HED} = BMDL_{05} \times (BW_a^{1/4}/BW_h^{1/4})$, BW_a = 0.25 kg, and BW_h = 70 kg.
[c]Based on toxicological equivalence of PBPK model derived AUC of RDX blood concentration.

Table D-25. Model predictions for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 5% ER

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1°[b] **Multistage 2°** **Multistage 3°** **Multistage 4°**	**0.493**	**49.095**	**28.5**	**11.8**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold. Scaled residuals for the selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were 0.89, −0.67, −0.74, 0.74, and −0.26, respectively.

[b]For the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

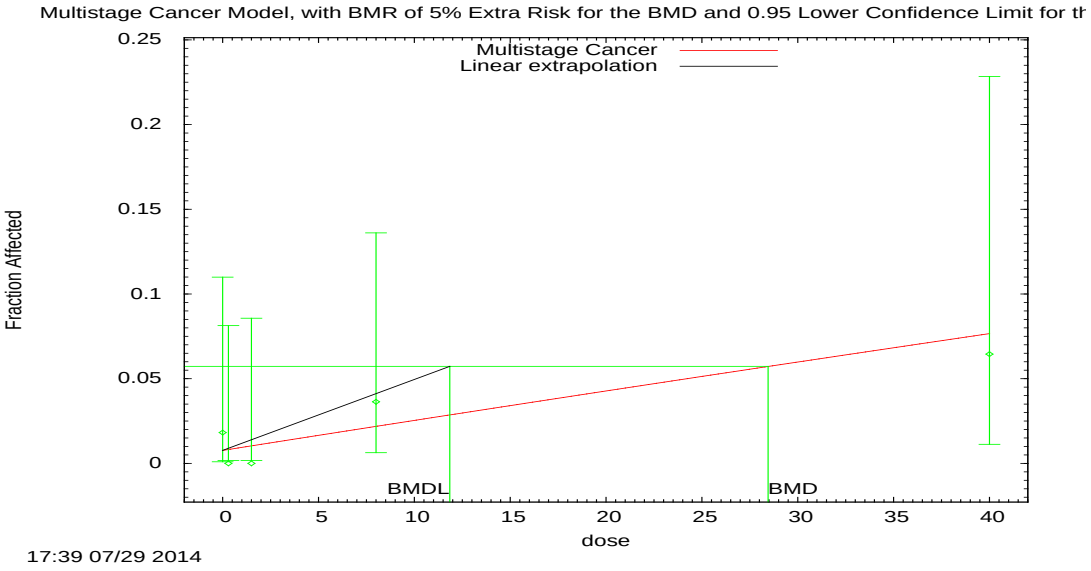


Figure D-18. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983).

Multistage Model (Version: 3.4; Date: 05/02/2014)

The form of the probability function is: P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2...)]

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 5% ER

BMD = 28.4525

BMDL at the 95% confidence level = 11.8487

BMDU at the 95% confidence level = 235.886

Taken together, (11.8487, 235.886) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00421987

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.00766363	0.00949438
Beta(1)	0.00180277	0.00149364

Analysis of Deviance Table

Model	Log (likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−21.0055	5			
Fitted model	−22.5473	2	3.08372	3	0.3789
Reduced model	−24.4692	1	6.92747	4	0.1398

AIC: = 49.0947

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0077	0.421	1	55	0.894
0.3	0.0082	0.451	0	55	−0.674
1.5	0.0103	0.538	0	52	−0.737
8	0.0219	1.203	2	55	0.735
40	0.0767	2.378	2	31	−0.255

Chi^2 = 2.4 d.f. = 3 *p*-value = 0.493

APPENDIX E. SUMMARY OF PUBLIC COMMENTS AND EPA's DISPOSITION

The Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was released for a 60-day public comment period on March 10, 2016. Public comments on the assessment were submitted to the U.S. Environmental Protection Agency (EPA) by the U.S. Army Public Health Command and Uniformed Services University of the Health Sciences (posted May 5, 2016), Johns Hopkins Bloomberg School of Public Health, Special Studies in Risk Assessment class (posted May 11, 2016), Ronald Melnick (posted May 19, 2016), and an anonymous member of the public (posted May 5, 2016). The anonymous public comment consisted of the word "good," and is not further discussed in this appendix.

A summary of major public comments provided in these submissions and EPA's response to these comments are provided in the sections that follow. The comments have been synthesized and paraphrased, and organized by topic and commenter. Editorial changes and factual corrections offered by public commenters were incorporated in the document as appropriate and are not discussed further. All public comments provided were taken into consideration in revising the draft assessment prior to release for external peer review. The complete set of public comments is available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-0430).

A public science meeting was held on May 10, 2016 to provide the public an opportunity to engage in early discussions on the draft Integrated Risk Information System (IRIS) toxicological review and the draft charge to the peer review panel prior to release for external peer review. The following three sets of slides were presented at the May 2016 public meeting on RDX and subsequently submitted to the RDX docket.

- Andy Nong (Health Canada) provided an overview of the physiologically based pharmacokinetic (PBPK) modeling of RDX that provided a framework for the discussion of modeling during the public science meeting. The slides did not provide specific comments on the IRIS assessment.

- Nancy Beck (American Chemistry Council) raised several broader programmatic topics concerning the Preamble and the use of quantitative analyses for chemicals with suggestive evidence of carcinogenicity. Beck also raised several issues specific to RDX, including further justification for the choice of a benchmark response (BMR) of 1% for convulsions and for selection of a gavage study (Crouse et al., 2006) over a dietary study as the basis for the reference dose (RfD). Questions related to both issues had been included in the charge to external peer reviewers.

- Larry Williams (formerly with U.S. Army Public Health Command) presented a slide titled "Realities of Human RDX Dose," suggesting that if an individual drank water containing RDX (at the chemical's water solubility), the toxicity of water would be greater than the toxicity of RDX. Williams identified the no-observed-adverse-effect level (NOAEL) in rats from the 90-day Crouse et al. (2006) study as 8 mg/kg-day, and estimated the corresponding equivalent dose in humans as 560 mg/kg-day (or approximately 0.5 g). Using a water solubility of RDX of 60 mg/L, Williams estimated that a 70-kg human would need to drink 9 L of RDX-saturated water to ingest 0.5 g RDX; he compared this value to a reported LD_{50} for water of 6 L.

  EPA identified the following issues with this analysis:

  - The dose of 8 mg/kg-day in the Crouse et al. (2006) study was not, in fact, a NOAEL, but rather a dose that caused convulsions in 15% of exposed rats and death in 10% of exposed rats.

  - Williams' analysis also failed to consider the statistical uncertainty around a NOAEL. In a study using 10 animals of each sex per dose group as in the Crouse et al. (2006) study, the 95% upper confidence limit on an observed response rate of 0% is 31%. Increasing the sample size to 20 animals per dose group by combining male and female rats would result in an upper confidence limit of 17%. Therefore, in this case, a response greater than 0% at the NOAEL is possible, even in the absence of statistical significance at that dose.

  - Williams' estimation of a human equivalent dose (HED) failed to take into consideration (1) possible differences in susceptibility between rodents and humans, including allometric scaling between rats and humans to account for species differences in toxicokinetics, and (2) potentially greater variation in sensitivity to RDX in the human population than in an in-bred strain of rat.

  In light of these omissions and misidentification of the NOAEL for RDX, the conclusion reached by Williams is not supported.

Comments Related to the Mechanisms by which RDX Induces Seizures

***Comment*:** On behalf of the U.S. Army Public Health Command and Uniformed Services University of the Health Sciences, Williams and colleagues submitted slides that summarized their research on the mechanism by which RDX induces seizures, including the measurement of in vivo acetylcholine and RDX concentrations in blood and brain samples following gavage exposure of rats, receptor binding assays, and in vitro extracellular and whole cell patch-clamp recordings. The authors concluded that (1) binding of RDX to the $GABA_A$ receptor convulsant site is the primary mechanism of seizure induction by RDX, (2) reduction of GABAergic inhibitory transmission in the rat basolateral amygdala is involved in RDX-induced seizures, and (3) the mechanism for RDX-induced seizures in rats is probably similar to humans. The submission provided no comments on the IRIS assessment of RDX.

***EPA Response*:** The submitted slide set summarizes the findings presented in the paper by Williams et al. (2011) published in Environmental Health Perspectives titled "RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: A mechanism for RDX-induced seizures." This paper is cited extensively by EPA in the discussion of mechanistic evidence for nervous system effects associated with RDX exposure (Section 1.2.1), and was considered an important primary source of information on the mechanism by which RDX induces seizures. The discussion of the potential mechanism of RDX-induced seizures in the RDX assessment is consistent with the Williams et al. (2011) paper and with the slides submitted to the docket.

Comments Related to the Cancer Descriptor

***Comment*:** Ronald Melnick commented that the cancer descriptor of *suggestive evidence of carcinogenic potential* was not supported, and that RDX clearly met the criteria for *likely to be carcinogenic to humans* according to the U.S. EPA (2005a) *Guidelines for Carcinogen Risk Assessment* (Cancer Guidelines) because RDX induced dose-related increases in tumors in two species (mouse and rat), in both sexes, and at two sites (liver and lung).

***EPA Response*:** As noted in the Cancer Guidelines (U.S. EPA, 2005a), "[c]hoosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence… Descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases that are clarified by the full narrative" (p. 2-51).

Interpretation of the evidence of carcinogenicity for RDX is not straight forward, and arguments for selecting more than one descriptor can be made. Section 1.3.2 of the public comment draft of the Toxicological Review had already presented the argument supported by Melnick for *likely to be carcinogenic to humans,* based on tumor findings in two species, both sexes, and two sites, as one of two plausible cancer descriptors, along with the argument for *suggestive evidence of carcinogenic potential*. The scientific support for the selection of the cancer descriptor for RDX had already been posed as a charge question to the Science Advisory Board's Chemical Assessment Advisory Committee (CAAC).

Melnick's assertion that EPA assigns cancer descriptors based on a set of criteria does not accurately characterize the selection of descriptors as discussed in the Cancer Guidelines. As noted in Section 2.5 of the Cancer Guidelines (p. 2-53), the bullets included under each cancer descriptor are examples that are illustrative of the combinations of evidence consistent with each of the five descriptors. As the Cancer Guidelines note, "[t]he examples are neither a checklist nor a limitation for the descriptor."

***Comment*:** Melnick supported his criticism of the selection of the *suggestive evidence of carcinogenic potential* descriptor with the observations that although the Toxicological Review identified statistically significant positive trends for hepatocellular adenomas or carcinomas (combined) in female mice and alveolar/bronchiolar carcinomas in male mice, it was incomplete by failing to note statistically significant increases in tumor incidence in individual dose groups based on pair-wise comparisons to the control.

***EPA Response*:** Melnick mischaracterized the role of statistical testing in evaluating evidence of an association between exposure and tumor response. In general, trend tests are preferred for evaluating response patterns across dose groups or exposure levels because they are more powerful in detecting overall dose-response trends than multiple pairwise comparisons to control. The presence of statistically significant pairwise comparisons to control does not provide additional information for assessing cancer hazard in this case.

Since trend tests often are not presented in study reports or journal articles (as is the case for RDX bioassays), EPA calculates trend tests where necessary. In Tables 1-13 and 1-14, the results of statistical analysis as reported by the authors are provided; EPA did not conduct statistical analyses using pairwise comparisons where the study authors did not, but did conduct the more informative trend tests as needed. In Section 1.3.2, EPA's evaluation of the carcinogenicity evidence for RDX intentionally relied on trend tests over pairwise comparisons. Thus, consideration of statistical analysis in Section 1.3.2 is not incomplete, but rather provides results of the more informative statistical tests.

***Comment*:** Melnick also offered the following comments on Section 1.3.2:

- The incidence of hepatocellular carcinomas in male rats, identified in the Toxicological Review as showing a statistically significant positive trend, should also have been compared to historical controls because it is a rare tumor in the F344 rat.

- It is inappropriate to emphasize the number of tumors in male rats in the mid- and high-dose groups in Levine et al. (1983) without adjusting for differences in the denominators in these groups.

- It is misleading to discuss the lack of tumor findings in the Hart (1976) study in Sprague-Dawley rats without discussing the limitations of that study.

***EPA Response*:** EPA agrees with these observations and revised Section 1.3.2 for greater transparency as follows:

- Text identifying hepatocellular carcinomas in male F344 rats as rare tumors was added to Section 1.3.2. Statistical comparison of the incidence of hepatocellular carcinomas in male rats in the RDX bioassay with historical control data from the National Toxicology Program had already been presented in Section 1.2.5 of the Toxicological Review.

- Text on the number of male rats with liver tumors was revised by providing the total number of animals examined. EPA agrees with including the denominator so that the tumor rate can be adjusted for the number of animals examined histopathologically. The incidence had already been provided in Table 1-13.

- Limitations of the Hart (1976) study had already been discussed in Section 1.2.5 and summarized in Section 1.3.2. Discussion of the limitations of the study in Section 1.3.2 was expanded to include that fact that examination of pathology in treated rats was limited to the high-dose group.

Comments submitted by the Johns Hopkins Bloomberg School of Public Health, Special Studies in Risk Assessment class

Comments were developed by the Special Studies in Risk Assessment class at the Johns Hopkins Bloomberg School of Public Health and submitted by Dr. Mary A. Fox, Assistant Professor and Acting Director of the Risk Sciences and Public Policy Institute. Selected major comments are summarized below.

***Comment*:** Planning and scoping for this review were not sufficiently explained. The assessment should include discussion of why RDX was selected for further study at this time, discussion of the specific public health concerns related to RDX (e.g., groundwater contamination, ingesting contaminated food), and whether there is concern that even small exposures to RDX could lead to illness. The assessment did not include information on past and present production quantities of RDX, geographic regional areas of use and distribution, summaries of potentially impacted populations, or demographic information (e.g., socio-economic status) that would assist in the evaluation of susceptible populations, support cumulative risk assessment activities, and help determine public health improvement metrics through proposed modification of the RfD.

***EPA Response*:** A planning and scoping step (introduced as part of the July 2013 enhancements to the IRIS process) was implemented well after the RDX assessment was initiated. Therefore, a formal planning and scoping step was not conducted for RDX; however, a brief discussion of environmental releases and occurrence, exposure potential, and regulatory interest that contributed to the selection of RDX for assessment development were provided in the Preface of the Toxicological Review. EPA notes that the mission of the IRIS Program is to identify and characterize the health hazards of chemicals, and that the scope of an IRIS assessment covers the first two steps of the risk assessment process: hazard identification and dose-response assessment. While the Preface includes discussion of uses and environmental occurrence, in general, exposure information falls outside the scope of an IRIS assessment.

Comment: More detailed explanation of criteria used for deciding which research to include or exclude in both hazard identification and selection of studies for dose-response modeling is needed. Specifically, the comment was offered that three rodent studies (MacPhail et al., 1985; Cholakis et al., 1980; Hart, 1976) were excluded in Section 1.2.1 because they showed no evidence of RDX-associated neurotoxicity, and explanation was sought for the aspects of these studies that made them unacceptable.

The Special Studies in Risk Assessment class also noted that three studies were selected as the basis for calculating candidate reference values for nervous system effects in Section 2.1.4, but the overall RfD for nervous system effects was based on a value derived from Crouse et al. (2006) rather than from Cholakis et al. (1980) that resulted in the lowest of the three values. They questioned that if the results of the Cholakis et al. (1980) study were going to be dismissed, why this study would have been selected as one of the key studies for RfD derivation purposes. It would be more appropriate that a study would either be dismissed during the principal study selection phase, with all of the appropriate evidence and justification, than after going through the motions of modeling and calculating benchmark doses (BMDs) and RfDs to ultimately dismiss the results anyway.

***EPA Response*:** The studies by MacPhail et al. (1985), Cholakis et al. (1980), and Hart (1976) were not excluded or determined to be unacceptable studies. Study evaluation was documented in the Literature Search Strategies | Study Selection and Evaluation section; studies determined to be uninformative were identified in that step and were not brought forward into hazard identification. MacPhail et al. (1985), Cholakis et al. (1980), and Hart (1976) were all included in Section 1.2 (Hazard Identification) as informative studies, and were considered in weighing the total evidence—positive and negative—for nervous system effects as a human hazard of RDX exposure in Section 1.2.1 (Integration of Nervous System Effects). Unlike the majority of toxicity studies of RDX, these three studies found no evidence of RDX-associated neurotoxicity. Section 1.2.1 provides a discussion of possible reasons that could account for a lack of nervous system response in these studies, but does not dismiss or exclude those findings.

In considering the comment concerned with bringing forward multiple studies for deriving candidate reference values, EPA points to Section 2.1.1, and particularly Table 2-1, that identifies factors considered in moving one or more studies forward for dose-response analysis. Among these are measurement of a representative outcome, reporting of incidence data, multiple dose groups and observation of a dose-related increase in the outcome, observation of an effect at a relatively low dose, and consideration of route of administration (e.g., diet or gavage). It is generally the case that studies considered for dose-response analysis have different strengths and limitations for estimating dose-response relationships; infrequently can a single "best" study be identified as the basis for a reference value. In bringing forward multiple studies for dose-response analysis, study strengths and limitations can be weighed in the context of the candidate values

derived from each study, and the influence of differences in study design (e.g., dose spacing, route of administration) and study quality can be examined. Similarities or differences in multiple candidate values from different studies can provide information on the confidence or uncertainty in the final reference value. Thus, performing dose-response analysis on datasets from multiple studies is more than "going through the motions of modeling"; rather, it informs selection of the overall reference value.

In the case of RDX, three studies of varying study design—Crouse et al. (2006), Cholakis et al. (1980), and Levine et al. (1983)—were selected for dose-response analysis of nervous system effects with the above considerations in mind. The rationale for selecting the candidate value based on Crouse et al. (2006) over candidate values from the other studies is provided in Section 2.1.4.

Comment: The public comments stated that justification of the hazard descriptor of *suggestive evidence of carcinogenic potential* was considered clear and sufficient. In another section of the comment document, however, the Special Studies in Risk Assessment class noted that it was not immediately clear how EPA applied the Cancer Guidelines, particularly the weight-of-evidence evaluation. Specifically, more detail was requested on specific factors that would increase or decrease weight of evidence, including the number of independent studies with consistent results, multiple observations across species/strain/site, route of administration (including the fact that the most common route of exposure in humans is inhalation but animal studies used oral administration; differences between species), and severity and progression.

***EPA Response*:** Several of the factors identified as contributing to the weight-of-evidence evaluation for carcinogenicity were already considered in Section 1.3.2 (Carcinogenicity). Other considerations, such as route of administration (only bioassays involving dietary exposure were available) did not influence the weight of evidence. As discussed in Section 1.3.2, the descriptor *suggestive evidence of carcinogenic potential* applies to all routes of human exposure, even where there is inadequate testing by an exposure route (i.e., inhalation exposure in the case of RDX), in the absence of convincing evidence to prove otherwise (see Cancer Guidelines, U.S. EPA, 2005a).

As noted in response to comments from Melnick, the charge to external peer reviewers already included a question as to whether the conclusion regarding weight of evidence for carcinogenicity was supported.

Comment: The limitations of gavage dosing studies, as well as why those limitations are of less concern than the limitations from the other studies, should be more clearly stated in Section 2.1.1.

***EPA Response*:** EPA recognizes the influence of method of dosing on the response to RDX in animal toxicity studies. Considerations related to the selection of a gavage study as the basis for the RfD over a dietary study were addressed in Sections 1.2.1 and 2.1.7, and this was identified as a key

issue in the Executive Summary. In addition, a question regarding the appropriateness of selecting Crouse et al. (2006), which used gavage administration, as the basis for the RfD is included in the charge to external peer reviewers.

REFERENCES FOR APPENDICES

Angerhofer, R; Davis, G; Balezewski, L. (1986). Teratological assessment of Trinitro - RDX in rats. (75-51-0573-86). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency.

Arfsten, DP; Davenport, R; Schaeffer, DJ. (1994). Reversion of bioluminescent bacteria (Mutatox TM) to their luminescent state upon exposure to organic compounds, munitions, and metal salts. Biomed Environ Sci 7: 144-149.

ATSDR (Agency for Toxic Substances and Disease Registry). (2012). Toxicological profile for RDX (Update) [ATSDR Tox Profile]. (NTIS/10590213). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bannon, DI. (2006). Toxicology study no. 87-XE-059F-06: Biomarkers of RDX exposure in breath of swine, August - September 2006. Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Bannon, DI; Dillman, JF; Hable, MA; Phillips, CS; Perkins, EJ. (2009a). Global gene expression in rat brain and liver after oral exposure to the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Chem Res Toxicol 22: 620-625. http://dx.doi.org/10.1021/tx800444k

Bannon, DI; Dillman, JF; Perkins, EJ; Bao, W; Wolfinger, RD; Chu, T; Phillips, CS. (2006). Acute RDX exposure and gene expression in the rat brain [Abstract]. Oxford, United Kingdom: Oxford University Press. http://www.toxicology.org/pubs/docs/Tox/2006Tox.pdf

Bannon, DI; Johnson, M; Williams, L; Adams, V; Perkins, E; Gust, K; Gong, P. (2009b). RDX and miRNA expression in B6C3F1 mice [Letter]. Environ Health Perspect 117: A98. http://dx.doi.org/10.1289/ehp.0800276

Barsotti, M; Crotti, G. (1949). [Attacchi epileptici come manifestazione di intossicazione professionale da trimetilen-trinitroamina (T4)]. Med Lav 40: 107-112.

Bhushan, B; Trott, S; Spain, JC; Halasz, A; Paquet, L; Hawari, J. (2003). Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a rabbit liver cytochrome P450: Insight into the mechanism of RDX biodegradation by Rhodococcus sp. strain DN22. Appl Environ Microbiol 69: 1347-1351. http://dx.doi.org/10.1128/AEM.69.3.1347-1351.2003

Brown, RP; Delp, MD; Lindstedt, SL; Rhomberg, LR; Beliles, RP. (1997). Physiological parameter values for physiologically based pharmacokinetic models [Review]. Toxicol Ind Health 13: 407-484. http://dx.doi.org/10.1177/074823379701300401

Cao, CJ; Reddy, G; Bannon, DI; Johnson, MS. (2008). In vitro study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) metabolism in human liver. Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-C-8027). Kansas City, MO: Midwest Research Institute.

Cotruvo, JA; Simmon, VF; Spanggord, RJ. (1977). Investigation of mutagenic effects of products of ozonation reactions in water. Ann N Y Acad Sci 298: 124-140. http://dx.doi.org/10.1111/j.1749-6632.1977.tb19259.x

Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Subchronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Crouse, LCB; Michie, MW; Major, MA; Leach, GJ; Reddy, G. (2008). Oral bioavailability of cyclotrimethylenetrinitramine (RDX) from contaminated site soils in rats. Int J Toxicol 27: 317-322. http://dx.doi.org/10.1080/10915810802366885

Davies, JOJ; Roberts, DM; Hittarage, A; Buckley, NA. (2007). Oral C-4 plastic explosive in humans - A case series. Clin Toxicol 45: 454-457. http://dx.doi.org/10.1080/15563650601118044

Dilley, JV; Tyson, CA; Newell, GW. (1979). Mammalian toxicological evaluation of TNT wastewaters. Volume II. Acute and subacute mammalian toxicity of TNT and the LAP mixture. (ADA080957. DAMD17-76-C-6050.). Menlo Park, CA: SRI International. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA080957

Fellows, RJ; Driver, CR; Cataldo, DA; Harvey, SD. (2006). Bioavailability of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the prairie vole (Microtus ochrogaster). Environ Toxicol Chem 25: 1881-1886. http://dx.doi.org/10.1897/05-446R1.1

George, SE; Huggins-Clark, G; Brooks, LR. (2001). Use of a Salmonella microsuspension bioassay to detect the mutagenicity of munitions compounds at low concentrations. Mutat Res 490: 45-56. http://dx.doi.org/10.1016/S1383-5718(00)00150-9

Goldberg, DJ; Green, ST; Nathwani, D; McMenamin, J; Hamlet, N; Kennedy, DH. (1992). RDX intoxication causing seizures and a widespread petechial rash mimicking meningococcaemia. J R Soc Med 85: 181.

Guo, L; Xu, H; Chen, Y; Chang, Y. (1985). Distribution and metabolism of tritium-labeled hexogen in white mice. 3: 335-339.

Harrell-Bruder, B; Hutchins, KL. (1995). Seizures caused by ingestion of composition C-4. Ann Emerg Med 26: 746-748. http://dx.doi.org/10.1016/S0196-0644(95)70049-8

Hart, ER. (1974). Subacute toxicity of RDX and TNT in dogs. Final report. (A717530). Kensington, MD: Litton Bionetics, Inc. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA035717

Hart, ER. (1976). Two-year chronic toxicity study in rats. (N00014-73-C-0162). Kensington, MD: Litton Bionetics, Inc. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA040161

Hathaway, JA; Buck, CR. (1977). Absence of health hazards associated with RDX manufacture and use. J Occup Med 19: 269-272.

Hess-Ruth, A; Crouse, L; Roszell, L. (2007). RDX pilot development neurotoxicity test in rats. (Toxicology Study No. 85-XC-064Y-07). Aberdeen Proving Ground: U.S. Army Center for Health Promotion and Preventive Medicine.

Hett, DA; Fichtner, K. (2002). A plastic explosive by mouth. J R Soc Med 95: 251-252. http://dx.doi.org/10.1258/jrsm.95.5.251

Hollander, AI; Colbach, EM. (1969). Composition C-4 induced seizures: A report of five cases. Mil Med 134: 1529-1530.

Johnson, MS. (2015). Memorandum for National Center for Environmental Assessment (8101R/Dr. Louis D'Amico), U.S. EPA - Office of Research and Development, 1200 Pennsylvania Ave., NW, Washington, DC 20460. Subject: Additional data from the oral subchronic toxicity of RDX in rats, 2006. (Toxicology study no. 85-XC-5131-03), U.S. Army Center for Health Promotion and Preventative Medicine, Aberdeen Proving Ground, Maryland; Incidence of seizure relative to mortality events. (MCHB-IP-T). Aberdeen Proving Ground, MD: Department of the Army, US Army Institute of Public Health.

Kaplan, AS; Berghout, CF; Peczenik, A. (1965). Human intoxication from RDX. Arch Environ Health 10: 877-883.

Kasuske, L; Schofer, JM; Hasegawa, K. (2009). Two marines with generalized seizure activity. J Emerg Nurs 35: 542-543. http://dx.doi.org/10.1016/j.jen.2008.05.001

Ketel, WB; Hughes, JR. (1972). Toxic encephalopathy with seizures secondary to ingestion of composition C-4. A clinical and electroencephalographic study. Neurology 22: 871-876.

Knepshield, JH; Stone, WJ. (1972). Toxic effects following ingestion of C-4 plastic explosive. In W Keup (Ed.), Drug Abuse: Current Concepts and Research (pp. 296-300). Springfield, IL: Charles C. Thomas.

Krishnan, K; Crouse, LCB; Bazar, MA; Major, MA; Reddy, G. (2009). Physiologically based pharmacokinetic modeling of cyclotrimethylenetrinitramine in male rats. J Appl Toxicol 29: 629-637. http://dx.doi.org/10.1002/jat.1455

Küçükardali, Y; Acar, HV; Özkan, S; Nalbant, S; Yazgan, Y; Atasoyu, EM; Keskin, O; Naz, A; Akyatan, N; Gökben, M; Danaci, M. (2003). Accidental oral poisoning caused by RDX (cyclonite): A report of 5 cases. J Intensive Care Med 18: 42-46. http://dx.doi.org/10.1177/0885066602239123

Lachance, B; Robidoux, PY; Hawari, J; Ampleman, G; Thiboutot, S; Sunahara, GI. (1999). Cytotoxic and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro. Mutat Res 444: 25-39. http://dx.doi.org/10.1016/S1383-5718(99)00073-X

Levin, DE; Hollstein, M; Christman, MF; Schwiers, EA; Ames, BN. (1982). A new Salmonella tester strain (TA102) with AT base pairs at the site of mutation detects oxidative mutagens. Proc Natl Acad Sci USA 79: 7445-7449. http://dx.doi.org/10.1073/pnas.79.23.7445

Levine, BS; Furedi, EM; Gordon, DE; Barkley, JJ; Lish, PM. (1990). Toxic interactions of the munitions compounds TNT and RDX in F344 rats. Fundam Appl Toxicol 15: 373-380. http://dx.doi.org/10.1016/0272-0590(90)90062-O

Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM. (1981a). Thirteen week oral (diet) toxicity study of trinitrotoluene (TNT), hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) and TNT/RDX mixtures in the Fischer 344 rat. Final report. (ADA108447. DAMD17-79-C-9120. DAMD17-79-C-9161). Chicago, IL: IIT Research Institute. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA108447

Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM. (1981b). Thirteen week toxicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 rats. Toxicol Lett 8: 241-245. http://dx.doi.org/10.1016/0378-4274(81)90108-9

Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM. (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report--phase V. Chicago, IL: IIT Research Institute.

Lipscomb, JC; Poet, TS. (2008). In vitro measurements of metabolism for application in pharmacokinetic modeling [Review]. Pharmacol Ther 118: 82-103. http://dx.doi.org/10.1016/j.pharmthera.2008.01.006

Lish, PM; Levine, BS; Furedi, EM; Sagartz, JM; Rac, VS. (1984). Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes1-3). (ADA181766. DAMD17-79-C-9161). Fort Detrick, Frederick, MD: U.S. Army Medical Research and Development Command. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA181766

Ma, B; Li, H. (1993). Neurobehavioral effects of hexogen on exposed workers. Gongye Weisheng yu Zhiyebin 19: 20-23.

MacPhail, RC; Walker, QD; Cook, L. (1985). Neurotoxicology of cyclotrimethylenetrinitramine (RDX). Final report. (ADA168266). Research Triangle Park, NC: U.S. Environmental Protection Agency, Health Effects Research Laboratory, Neurotoxicology Division. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA168266

Major, MA; Reddy, G; Berge, MA; Patzer, SS; Li, AC; Gohdes, M. (2007). Metabolite profiling of [14C]hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. J Toxicol Environ Health A 70: 1191-1202. http://dx.doi.org/10.1080/15287390701252717

Martin, D; Hart, E. (1974). Subacute toxicity of RDX and TNT in monkeys (pp. 1-216). (ADA044650). Kensington, MD: Litton Bionetics, Inc. https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA044650

Merrill, SL. (1968). Ingestion of an explosive material, composition C-4: A report of two cases. USARV Med Bull 40: 5-11.

Musick, TJ; Berge, MA; Patzer, SS; Tilch, KR. (2010). Absorption, distribution, metabolism, and excretion of 14C-RDX following oral administration to minipigs (DAAD05-02-P-2319). (ADA526472). Madison, WI: Covance Laboratories Inc. http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA526472

Neuwoehner, J; Schofer, A; Erlenkaemper, B; Steinbach, K; Hund-Rinke, K; Eisentraeger, A. (2007). Toxicological characterization of 2,4,6-trinitrotoluene, its transformation products, and two nitramine explosives. Environ Toxicol Chem 26: 1090-1099. http://dx.doi.org/10.1897/06-471R.1

NIOSH (National Institute for Occupational Safety and Health). (2012). NIOSH pocket guide to chemical hazards: Cyclonite, CAS 121-82-4. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/npg/npgd0169.html

OSHA (Occupational Safety & Health Administration). (2012a). Air contaminants. Table Z-Shipyards. CFR 29 CFR 1915, Subpt. P, App A.

OSHA (Occupational Safety & Health Administration). (2012b). Gases, vapors, fumes, dusts, and mists. Appendix A to 1926.55-1970 American Conference of Governmental Industrial Hygienists' threshold limit values of airborne contaminants. CFR 29 CFR 1926.55.

Özhan, G; Topuz, S; Alpertunga, B. (2003). Determination of cyclonite (RDX) in human plasma by high-performance liquid chromatography. Farmaco 58: 445-448. http://dx.doi.org/10.1016/S0014-827X(03)00069-7

Pan, X; Ochoa, KM; San Francisco, MJ; Cox, SB; Dixon, K; Anderson, TA; Cobb, GP. (2013). Absorption, distribution, and biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in B6C3F1 mice (Mus musculus). Environ Toxicol Chem 32: 1295-1303. http://dx.doi.org/10.1002/etc.2188

Pan, X; San Francisco, MJ; Lee, C; Ochoa, KM; Xu, X; Liu, J; Zhang, B; Cox, SB; Cobb, GP. (2007a). Examination of the mutagenicity of RDX and its N-nitroso metabolites using the Salmonella reverse mutation assay. Mutat Res Genet Toxicol Environ Mutagen 629: 64-69. http://dx.doi.org/10.1016/j.mrgentox.2007.01.006

Pan, X; Zhang, B; Smith, JN; San Francisco, M; Anderson, TA; Cobb, GP. (2007b). N-Nitroso compounds produced in deer mouse (Peromyscus maniculatus) GI tracts following hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure. Chemosphere 67: 1164-1170. http://dx.doi.org/10.1016/j.chemosphere.2006.10.077

Paquet, L; Monteil-Rivera, F; Hatzinger, PB; Fuller, ME; Hawari, J. (2011). Analysis of the key intermediates of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) in groundwater: Occurrence, stability and preservation. J Environ Monit 13: 2304-2311. http://dx.doi.org/10.1039/c1em10329f

Parker, GA; Reddy, G; Major, MA. (2006). Reevaluation of a twenty-four-month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse. Int J Toxicol 25: 373-378. http://dx.doi.org/10.1080/10915810600846245

Poulin, P; Krishnan, K. (1995). An algorithm for predicting tissue : blood partition coefficients of organic chemicals from n-octanol : water partition coefficient data. J Toxicol Environ Health 46: 117-129. http://dx.doi.org/10.1080/15287399509532021

Poulin, P; Theil, F, -P. (2000). A priori prediction of tissue : plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. J

Pharm Sci 89: 16-35. http://dx.doi.org/10.1002/(SICI)1520-6017(200001)89:1<16::AID-JPS3>3.0.CO;2-E

Reddy, G; Allen, NA; Major, MA. (2008). Absorption of 14C-cyclotrimethylenetrinitramine (RDX) from soils through excised human skin. Toxicol Mech Meth 18: 575-579. http://dx.doi.org/10.1080/15376510701703466

Reddy, G; Eisenhut, K; Morrison, SA; Kelly, JA. (1989). Toxicokinetics of 14C RDX (cyclotrimethylenetrinitramine) in rats after intratracheal administration [Abstract].

Reddy, G; Erexson, GL; Cifone, MA; Major, MA; Leach, GJ. (2005). Genotoxicity assessment of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Int J Toxicol 24: 427-434. http://dx.doi.org/10.1080/10915810500366922

Reifenrath, WG; Kammen, HO; Reddy, G; Major, MA; Leach, GJ. (2008). Interaction of hydration, aging, and carbon content of soil on the evaporation and skin bioavailability of munition contaminants. J Toxicol Environ Health A 71: 486-494. http://dx.doi.org/10.1080/15287390801906956

Rispin, A; Farrar, D; Margosches, E; Gupta, K; Stitzel, K; Carr, G; Greene, M; Meyer, W; McCall, D. (2002). Alternative methods for the median lethal dose (LD(50)) test: The up-and-down procedure for acute oral toxicity. ILAR J 43: 233-243. http://dx.doi.org/10.1093/ilar.43.4.233

Safe Work Australia. (2014). Hazardous substances information system (HSIS). Exposure standard documentation: Cyclonite [Database]. Retrieved from http://www.hsis.safeworkaustralia.gov.au/ExposureStandards/Document?exposureStandardID=174

Schneider, NR; Bradley, SL; Andersen, ME. (1977). Toxicology of cyclotrimethylenetrinitramine: Distribution and metabolism in the rat and the miniature swine. Toxicol Appl Pharmacol 39: 531-541. http://dx.doi.org/10.1016/0041-008X(77)90144-2

Schneider, NR; Bradley, SL; Andersen, ME. (1978). The distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in the rat after subchronic administration. Toxicol Appl Pharmacol 46: 163-171. http://dx.doi.org/10.1016/0041-008X(78)90147-3

Simmon, VF; Spanggord, RJ; Eckford, SL; McClurg, V. (1977). Mutagenicity of some munition wastewater chemicals and chlorine test kit reagents (pp. 1-205). (ADA057680. DAMD17-76-C-6013). Menlo Park, CA: SRI International. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA057680

Smith, JN; Espino, MA; Liu, J; Romero, NA; Cox, SB; Cobb, GP. (2009). Multigenerational effects in deer mice (Peromyscus maniculatus) exposed to hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). Chemosphere 75: 910-914. http://dx.doi.org/10.1016/j.chemosphere.2009.01.010

Snodgrass, HL, Jr. (1984). Preliminary assessment of relative toxicity and mutagenicity potential of 1-nitroso-3,5-dinitro-1,3,5-triazacylcohexane (mononitroso-RDX) (Final Report ed.). (ADA149351. USAEHA-75-51-0345-85). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA149351

Snoeck, V; Huyghebaert, N; Cox, E; Vermeire, A; Saunders, J; Remon, JP; Verschooten, F; Goddeeris, BM. (2004). Gastrointestinal transit time of nondisintegrating radio-opaque pellets in suckling and recently weaned piglets. J Control Release 94: 143-153. http://dx.doi.org/10.1016/j.jconrel.2003.09.015

Spiteri, NJ. (1982). Circadian patterning of feeding, drinking and activity during diurnal food access in rats. Physiol Behav 28: 139-147. http://dx.doi.org/10.1016/0031-9384(82)90115-9

Stone, WJ; Paletta, TL; Heiman, EM; Bruce, JI; Knepshield, JH. (1969). Toxic effects following ingestion of C-4 plastic explosive. Arch Intern Med 124: 726-730. http://dx.doi.org/10.1001/archinte.1969.00300220078015

Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review]. Regul Toxicol Pharmacol 62: 107-114. http://dx.doi.org/10.1016/j.yrtph.2011.12.007

Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64: 205-224. http://dx.doi.org/10.1016/j.yrtph.2012.07.005

Tan, EL; Ho, CH; Griest, WH; Tyndall, RL. (1992). Mutagenicity of trinitrotoluene and its metabolites formed during composting. J Toxicol Environ Health A 36: 165-175. http://dx.doi.org/10.1080/15287399209531632

Testud, F; Glanclaude, J, -M; Descotes, J. (1996a). Acute hexogen poisoning after occupational exposure. J Toxicol Clin Toxicol 34: 109-111. http://dx.doi.org/10.3109/15563659609020244

Testud, F; Glanclaude, J; Imperatori, J; Le Meur, B; Descotes, J. (1996b). [Acute hexogen poisoning after occupational exposure, report of 2 cases]. Arch Mal Prof Med Trav Secur Soc 57: 342-346.

Timchalk, C; Nolan, RJ; Mendrala, AL; Dittenber, DA; Brzak, KA; Mattsson, JL. (2002). A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci 66: 34-53. http://dx.doi.org/10.1093/toxsci/66.1.34

U.S. EPA (U.S. Environmental Protection Agency). (1992). A cross-species scaling factor for carcinogen risk assessment based on equivalence of mg/kg3/4/day [EPA Report] (pp. 24152-24161). Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www2.epa.gov/osa/guidelines-carcinogen-risk-assessment

U.S. EPA (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (pp. 1-125). (EPA/630/R-03/003F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S. EPA (U.S. Environmental Protection Agency). (2012a). Advances in inhalation gas dosimetry for derivation of a reference concentration (RfC) and use in risk assessment (pp. 1-140). (EPA/600/R-12/044). Washington, DC. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=244650&CFID=50524762&CFTOKEN=17139189

U.S. EPA (U.S. Environmental Protection Agency). (2012b). Benchmark dose technical guidance (pp. 1-99). (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

U.S. EPA (U.S. Environmental Protection Agency). (2012c). EPA Federal facilities forum issue paper: Site characterization for munitions constituents [EPA Report] (pp. 1-170). (EPA-505-S-11-001). Washington, D.C. http://www2.epa.gov/fedfac/epa-federal-facilities-forum-issue-paper-site-characterization-munitions-constituents

U.S. EPA (U.S. Environmental Protection Agency). (2014). RDX PBPK files in acsIX format [EPA Report].

von Oettingen, WF; Donahue, DD; Yagoda, H; Monaco, AR; Harris, MR. (1949). Toxicity and potential dangers of cyclotrimethylenetrinitramine (RDX). J Ind Hyg Toxicol 31: 21-31.

West, RR; Stafford, DA. (1997). Occupational exposures and haematological abnormalities among ordnance factory workers: A case control study. Leuk Res 21: 675-680. http://dx.doi.org/10.1016/S0145-2126(97)00042-8

Whong, W, -Z; Speciner, ND; Edwards, GS. (1980). Mutagenic activity of tetryl, a nitroaromatic explosive, in three microbial test systems. Toxicol Lett 5: 11-17. http://dx.doi.org/10.1016/0378-4274(80)90142-3

Williams, LR; Aroniadou-Anderjaska, V; Qashu, F; Finne, H; Pidoplichko, V; Bannon, DI; Braga, MFM. (2011). RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: A mechanism for RDX-induced seizures. Environ Health Perspect 119: 357-363. http://dx.doi.org/10.1289/ehp.1002588

Woody, RC; Kearns, GL; Brewster, MA; Turley, CP; Sharp, GB; Lake, RS. (1986). The neurotoxicity of cyclotrimethylenetrinitramine (RDX) in a child: A clinical and pharmacokinetic evaluation. Clin Toxicol 24: 305-319. http://dx.doi.org/10.3109/15563658608992595