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

(CASRN 121-82-4)

Supplemental Information

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Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
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DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or 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
AUC_{Total}	area under the curve for blood concentration versus time from the time of doxing to the time RDX is completely eliminated
BDNF	brain-derived neurotrophic factor
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
$BW^{0.33}$	body weight scaling to the 0.33 power
$BW^{2/3}$	body weight scaling to the 2/3 power
$BW^{3/4}$	body weight scaling to the ¾ power
BW_a	animal body weight
BW_h	human body weight
CAAC	Chemical Assessment Advisory Committee
CASRN	Chemical Abstracts Service registry number
CI	confidence interval
C_{max}	peak concentration
CNS	central nervous system
CSF	cerebrospinal fluid
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
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
EPA	Environmental Protection Agency
ER	extra risk
FOB	functional observational battery
FUDS	Formerly Used Defense Sites
GABA	gamma-amino butyric acid
GD	gestational day
GI	gastrointestinal
HED	human equivalent dose
HERO	Health and Environmental Research Online
HI	hazard index
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
KAD	rate constant for oral absorption, compartment 2
KAS	rate constant for oral absorption, compartment 1
Kel	terminal elimination rate constant
KfC	metabolic rate constant
KQB	fractional blood flow to brain
KQC	cardiac output
KQF	fractional blood flow to fat
KQL	fractional blood flow to liver
KQR	fractional blood flow to richly perfused tissue
KQS	fractional blood flow to slowly perfused tissue
KVB	fractional tissue volume of brain
KVF	fractional tissue volume of fat
KVL	fractional tissue volume of liver
KVR	fractional tissue volume of richly perfused tissue
KVS	fractional tissue volume of slowly perfused tissue
KVV	fractional tissue volume of blood volume
LD_{01}	the dose expected to be lethal to 1% of the animals
LDH	lactate dehydrogenase
LDL_{01}	lower confidence limit on the LD_{01}
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
NA	not available
NCE	normochromatic erythrocyte

NCEA	National Center for Environmental Assessment
ND	not determined
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
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
PB	tissue:blood partition coefficient for brain
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocyte
PECO	populations, exposures, comparitors, and outcomes
PEL	permissible exposure limit
PF	tissue:blood partition coefficient for fat
PL	tissue:blood partition coefficient for liver
PND	postnatal day
POD	point of departure
PR	tissue:blood partition coefficient for richly perfused tissue
PS	tissue:blood partition coefficient for slowly perfused tissue
PWG	Pathology Working Group
RBC	red blood cell
RDX	Royal Demolition eXplosive (hexahydro-1,3,5-trinitro-1,3,5-triazine)
RfC	inhalation reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
SAB	Science Advisory Board
SD	standard deviation
SDMS	spontaneous death or moribund sacrifice
SDWA	Safe Drinking Water Act
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SLE	systemic lupus erythematosus
SS	scheduled sacrifice
t½	half life
TLV	threshold limit value
TNT	trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
TS	terminal sacrifice
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

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 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 [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 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—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 OSHA-proposed PEL in 1988 PEL hearings Skin designation indicates potential for dermal absorption Basis: Agreed with OSHA's proposal for skin notation in 1988 PEL hearings
Occupational Safety and Health Administration (OSHA, 2012)	PEL—1.5 mg/m^3 TWA for an 8-hr workday in a 40-hr workweek Basis: Adopted from the ACGIH 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 Chemical Information System (Safe Work Australia, 2018)	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

ACGIH = American Conference of Governmental Industrial Hygienists; MRL = minimal risk level; OSHA = Occupational Safety and Health Administration; PBPK = physiologically based pharmacokinetic; PEL = permissible exposure limit; TLV = threshold limit value; TWA = time-weighted average; UF = uncertainty factor.

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, Toxic Substances Control Act Test Submissions (TSCATS), and Toxcenter—is provided in Table B-1. Toxcenter, a fee-based scientific database, was searched outside of Health and Environmental Research Online (HERO).[1] 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 (see 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.

A post-peer-review literature search update was conducted in PubMed, Toxline, and TSCATS for the period May 2016 to November 2017 and in DTIC for the period 2016 to February 2018 using a search strategy consistent with previous literature searches (see Tables B-1 and B-2).

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

[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 by the National Center for Environmental Assessment. The database includes more than 1.6 million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO.

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 either 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 were 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). A second update of the DTIC search was performed in February 2018, after peer review of the RDX assessment. Included records were those with creation dates of January 2016 to February 2018. The update search identified 46 items classified as "approved for public release," 9 classified as "distribution limited to U.S. government agencies and their contractors," and 13 classified as "distribution limited to U.S. government agencies only"; none of these were selected for further review because none met the inclusion criteria outlined in Table LS-1 of the main document or they were duplicates of references already cited in the RDX assessment.

The 85 unique selected citations with unlimited distribution from the January 2015 search were uploaded to the HERO website (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.

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (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	337

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
PubMed Date: 4/2012 (continued)	"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]))))) AND (invertebrates OR aquatic organisms OR fish OR fishes OR amphibians OR earthworm*))	

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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
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

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
PubMed Date limit: 5/2016–11/2017 *(post-peer review)*	("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 (2016/05/01 : 3000)	229
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

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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+@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	0

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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
Toxline Date limit: 5/2016–11/2017 *(post-peer review)*	@BOOL+("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")+@RANGE+yr+2016+2017+@NOT+@org+pubmed+pubdart+crisp+tscats+nih @BOOL+("cyclonite" 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 "Hexogenor"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")+@RANGE+yr+2016+2017+@NOT+@org+pubmed+pubdart+crisp+tscats+nih	0
TSCATS Date: 2/2013	@term+@rn+121-82-4+@AND+@org+tscats	4

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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
TSCATS Date: 11/2017 *(post-peer review)*	Not searched. TSCATS is no longer being updated.	--

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

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)

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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 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.	26 titles screened (6 selected for full records and added to HERO)

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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 (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.	20 titles screened (0 selected for full records; none added to HERO)

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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? 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.	80 titles screened (3 selected for full records and added to HERO)

Table B-1. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (PubMed, Toxline, Toxcenter, TSCATS) (continued)

Database	Terms	Hits
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)) Note: Duplicates were removed.	33 titles screened (0 selected for full records and added to HERO)

CASRN = Chemical Abstracts Registry Number; CI = confidence interval.

Table B-2. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (DTIC online database)

Date accessed	Terms	Hits
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. government and contractors	239 (0 selected and added to HERO)
	Distribution: U.S. government only	199 (0 selected and added to HERO)
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	
	Distribution: Approved for public release	21 (0 selected and added to HERO)
	Distribution: U.S. government and contractors	9 (0 selected and added to HERO)

Table B-2. Summary of detailed search strategies for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (DTIC) (continued)

Database	Terms	Hits
5/2016 (continued)	Distribution: U.S. government only	9 (0 selected and added to HERO)
2/16/2018 (*post-peer review*)	"cyclonite" 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 108E" OR "PbxAF 108" Limited to content type: Documents *Includes records with creation dates of January 2016 to February 2018.*	
	Distribution: Approved for public release	46 (0 selected and added to HERO)
	Distribution: U.S. government and contractors	9 (0 selected and added to HERO)
	Distribution: U.S. government only	13 (0 selected and added to HERO)

Table B-3. Processes used to augment the search of core databases for hexahydro-1,3,5-trinitro-1,3,5-triazine (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 noncancer 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 noncancer 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

Table B-3. Processes used to augment the search of core databases for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Selected key reference(s) or sources	Date	Additional references identified
Online regulatory sources		
Combination of CASRN and synonyms searched on the following websites: 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 (http://www.epa.gov/ncepihom/) EPA Science Inventory (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 (http://monographs.iarc.fr/htdig/search.html) International Programme on Chemical Safety INCHEM (http://www.inchem.org/) National Academy of Science via the National Academies Press (http://www.nap.edu/) National Cancer Institute (http://www.cancer.gov) National Center for Toxicological Research (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 NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) NTP—Report on Carcinogens, status, results, and management reports (http://ntpsearch.niehs.nih.gov/query.html) World Health Organization assessments—Concise International Chemical Assessment Documents, Environmental Health Criteria (http://www.who.int/ipcs/assessment/en/)	4/2012 1/2014 1/2015 5/2016	15 citations added 1 citation added 0 citations added 0 citations added

ATSDR = Agency for Toxic Substances and Disease Registry; CASRN = Chemical Abstracts Registry Number; NTP = National Toxicology Program; PBPK = physiologically based pharmacokinetic.

[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) by measuring 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 animal 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. No studies investigating absorption of RDX following inhalation exposure have been 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 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 (Woody et al., 1986).[2] Sweeney et al. (2012a) estimated the absorption rate constant for this same subject to be 0.060/hour. 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 contact and inhalation, 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. Küçükardali et al. (2003) summarized plasma RDX levels in another group of 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 included the GI tract, which may have included unabsorbed RDX. Assuming that all of the carbon-14 in feces represented 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 the 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 $t_{½}$ 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 hour-% 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 pigs (minipigs) 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 on 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, a result consistent with the greater surface area available for absorption with the finely powdered RDX. However, blood levels were only measured 24 hours after dosing, and the lower 2-hour carbon-14 plasma concentration for the coarse granular preparation could be due to slower absorption of the 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 comparing 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., 2009; 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 study authors estimated an absorption rate constant of 8.7/hour. 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., 2009; 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 Yorkshire piglets administered single doses (5–10 mg/kg) of finely powdered (>98% pure) RDX in gelatin capsules occurred at 3–8 hours after dosing (Bannon, 2006) compared to 24 hours after a single dose (100 mg/kg) of RDX administered as a fine powder in saline (Schneider et al., 1977).

Peak plasma concentrations in minipigs 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 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 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 (Pan et al., 2013; 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. Because 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 (see Table C-1) (Bannon, 2006; Schneider et al., 1977). With repeated oral dosing (e.g., 28–90 days), tissue:blood ratios of RDX for these tissues were consistently greater than unity (Pan et al., 2013; 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).

Plasma protein binding may be a factor impacting the concentration of RDX available to diffuse across the blood-brain barrier and to be metabolized. While empirical measurements of protein binding are not available, some of the toxicokinetic studies can be informative. The plasma:whole blood ratios measured in Musick et al. (2010) and Krishnan et al. (2009) are approximately 1.3 and 1.0, which are not suggestive of a high affinity for protein binding. The volume of distribution estimated in Schneider et al. (1977) is 2.18 L/kg, approximately twice body size, which is again not suggestive of a high affinity for protein binding. In the absence of evidence of protein binding, the total amount of RDX is considered potentially available for diffusion across the blood-brain barrier and for metabolism.

Table C-1. Distribution of hexahydro-1,3,5-trinitro-1,3,5-triazine (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[e]	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[f]	24	1[c]	0.8	1	1.4	0.8	Guo et al. (1985)
Mouse	i.v.	2.5[f]	24	0.6[g]	0.8	0.7	1.6	0.4	Guo et al. (1985)

NA = not available.
[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]Not available
[f]Tritium
[g]Tissue:blood

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

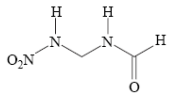
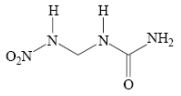
The metabolism of RDX is not well characterized. No studies investigating the metabolism of RDX in humans were identified. Studies in animals indicate that RDX undergoes extensive metabolism, including denitration, ring cleavage, and generation of CO_2. Predominant metabolic pathways and major organs 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 the nitrosamine RDX metabolites hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), and further metabolism to CO_2. In mice at the end of a 28-day exposure to RDX in feed (ad libitum), the nitrosamine RDX metabolites (MNX, DNX, and TNX) were measured (Pan et al., 2013). RDX and the metabolites MNX, DNX, and TNX were detected in the brain and other tissues. 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 the 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 minipigs 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 MNX, DNX, and TNX; methylene dinitramine and 4-nitro-2-diazbutanal have also been detected (Jaligama et al., 2013; Halasz et al., 2012; Sweeney et al., 2012b; Paquet et al., 2011; Fuller et al., 2010; Smith et al., 2006; Meyer et al., 2005; Beller and Tiemeier, 2002) (see additional discussion in Section 1.1.1 of the Toxicological Review). The toxicity of these environmental breakdown products has received little investigation [e.g., see Meyer et al. (2005) and Smith et al. (2006)].

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 minipigs, 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 minipigs 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 gavage studies with Yucatan minipigs, only trace amounts of the nitrosamine RDX metabolites MNX and 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 TNX. The study authors suggested that the trace levels of these metabolites 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 study 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 U.S. Environmental Protection Agency (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 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in minipigs 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 and rabbit liver CYP450 2B4 under anaerobic conditions produced nitrite, 4-nitro-2,4-diazabutanal, formaldehyde, and ammonium ions (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-aminobenzotriazole, 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 (see Table C-3). Woody et al. (1986) estimated the elimination $t_{½}$ to be approximately 15 hours in a child who ingested approximately 85 mg of RDX per kg of body weight. The $t_{½}$ 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 $t_{½}$ of 20–30 hours was derived (calculated for this review by fitting the serum RDX data to a first-order exponential function). It is not possible to draw reliable inferences from these values because they are based on accidental, acute exposures, and in particular, the data for the child are based on a single set of measurements of one individual.

Table C-3. Elimination $t_{½}$ values for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) or radiolabeled RDX

Animal	Route	Dose (mg/kg)	Time[a]	$t_{½}$ (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)

NA = not available.
[a]Observation period following exposure on which the $t_{½}$ 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 $t_{½}$ 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 $t_{½}$ of approximately 1.2 hours. The elimination $t_{½}$ estimated for rats (Krishnan et al., 2009; Schneider et al., 1977) is as much as an order of magnitude longer than in mice (Sweeney et al., 2012b).

C.1.5. Physiologically Based Pharmacokinetic (PBPK) Models

Overview of Available Physiologically Based Pharmacokinetic (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 (see 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, AL) was obtained from the study authors of Sweeney et al. (2012a).

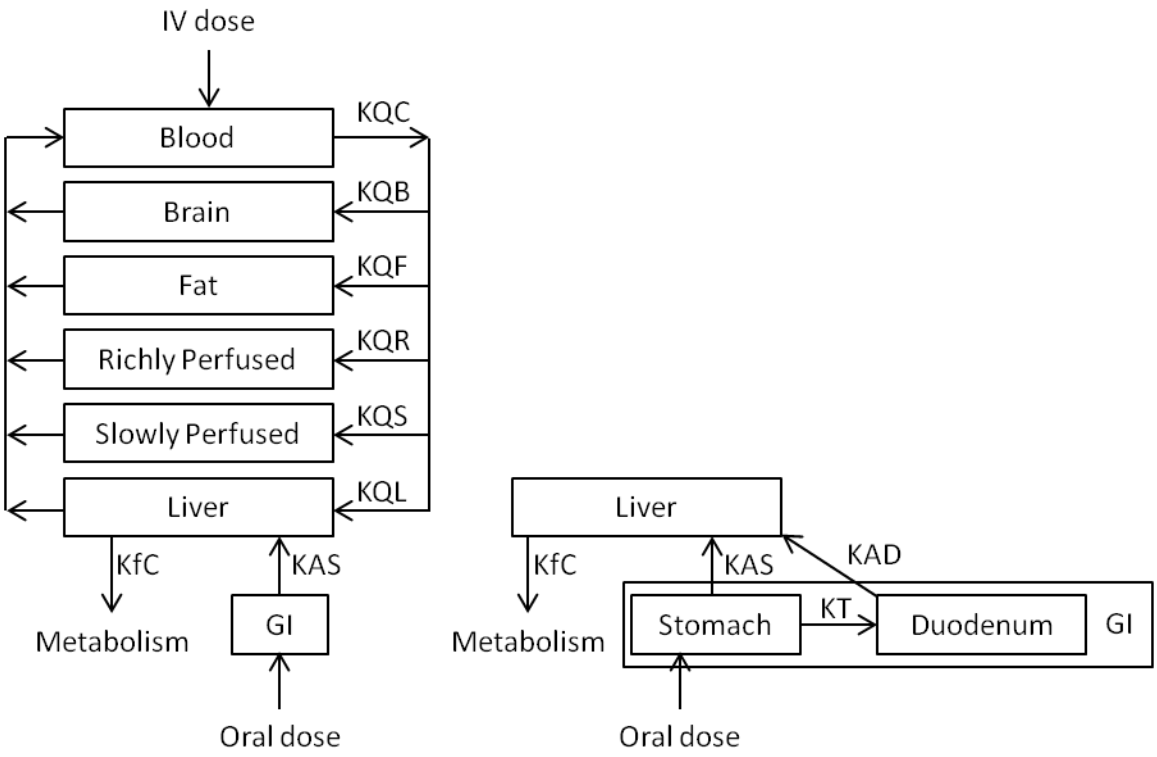


Figure C-1. Physiologically based pharmacokinetic (PBPK) model structure for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in rats and humans.[a]

[a]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).

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) physiologically based pharmacokinetic (PBPK) models for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in rats, humans, and mice as reported by the study authors

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
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)
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]

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

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
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]

BW = body weight: KAD = rate constant for oral absorption, compartment 2; KAS = rate constant for oral absorption, compartment 1; KQB = fractional blood flow to brain; KQC = cardiac output; KQF = fractional blood flow to fat; KQL = fractional blood flow to liver; KQR = fractional blood flow to richly perfused tissue; KQS = fractional blood flow to slowly perfused tissue; KVB = fractional tissue volume of brain; KVF = fractional tissue volume of fat; KVL = fractional tissue volume of liver; KVR = fractional tissue volume of richly perfused tissue; KVS = fractional tissue volume of slowly perfused tissue; KVV = fractional tissue volume of blood volume; NA = not available.

[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. (2009), 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 the RDX in capsules (Bannon et al., 2009; 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 (see 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 body weight scaling to the 0.33 power ($BW^{0.33}$)/ 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.

Physiologically Based Pharmacokinetic (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 (fractional tissue volume of richly perfused tissue [KVR] and fractional blood flow to slowly perfused tissue [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 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 (see 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
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)
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]

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

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
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

BW = body weight; KQB = fractional blood flow to brain; KQC = cardiac output; KQF = fractional blood flow to fat; KQL = fractional blood flow to liver; KQR = fractional blood flow to richly perfused tissue; KVB = fractional tissue volume of brain; KVF = fractional tissue volume of fat; KVL = fractional tissue volume of liver; KVS = fractional tissue volume of slowly perfused tissue; KVV = fractional tissue volume of blood volume.

[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).

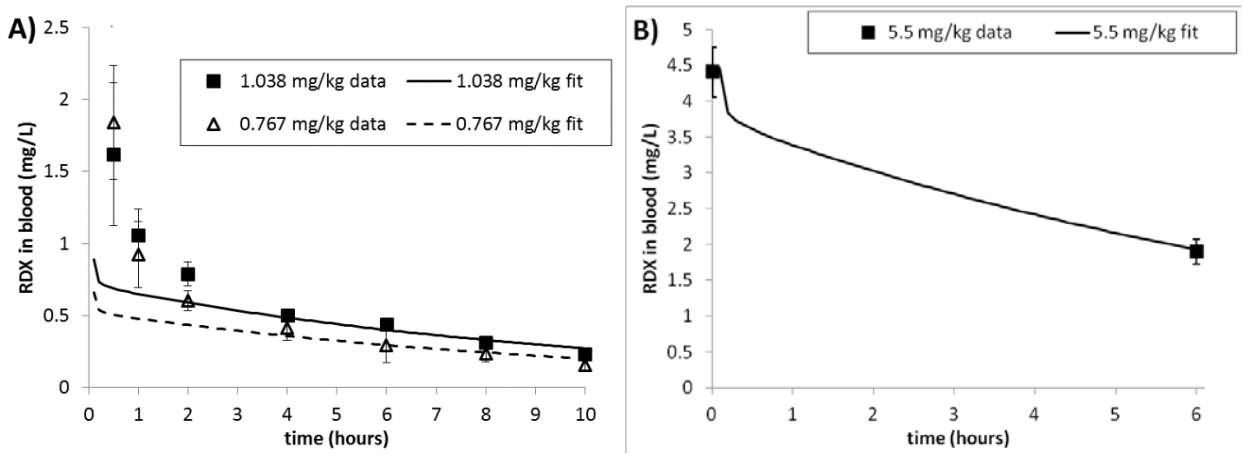


Figure C-2. EPA rat physiologically based pharmacokinetic (PBPK) model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations in male and female Sprague-Dawley rats following i.v. exposure.[a]

[a]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).

Absorption of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) from the gastrointestinal (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 (see 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. (2009) studies (see 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 (see Figure C-7). The Schneider et al. (1977) study was used to estimate the absorption rate constants for coarse-grain RDX (see 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 (see 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. As discussed in Section C.1.2, Distribution, plasma protein binding could be a factor impacting RDX diffusion across the blood-brain barrier. However, in the absence of empirical values for estimating protein binding, the total RDX blood concentration is assumed available for diffusion across the blood-brain barrier.

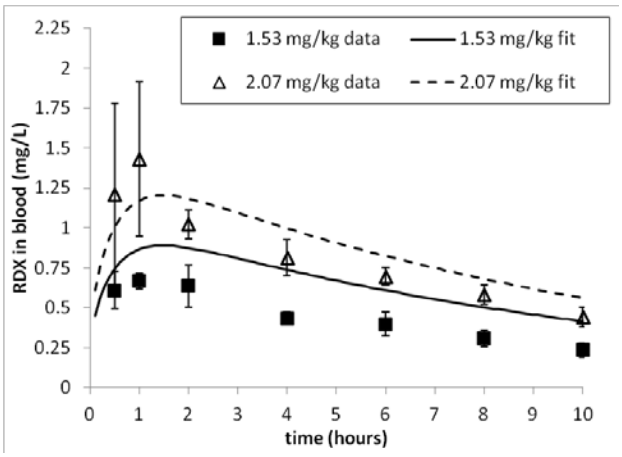


Figure C-3. EPA rat physiologically based pharmacokinetic (PBPK) model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations following oral exposure to RDX dissolved in water.[a]

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

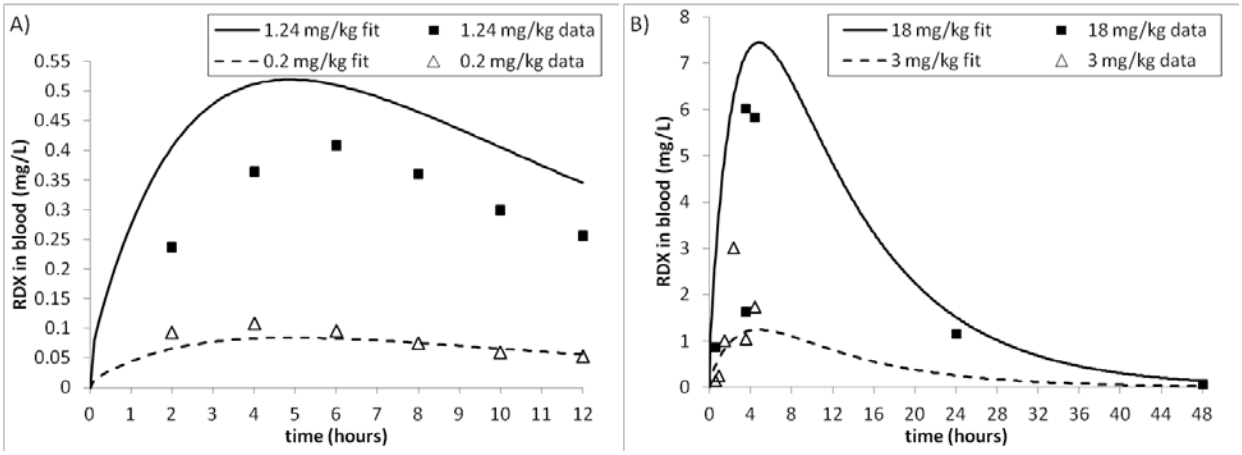


Figure C-4. EPA rat model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations following oral exposure to RDX in dry capsules.[a]

[a]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. (2009)] for KAS = 0.35/hour.

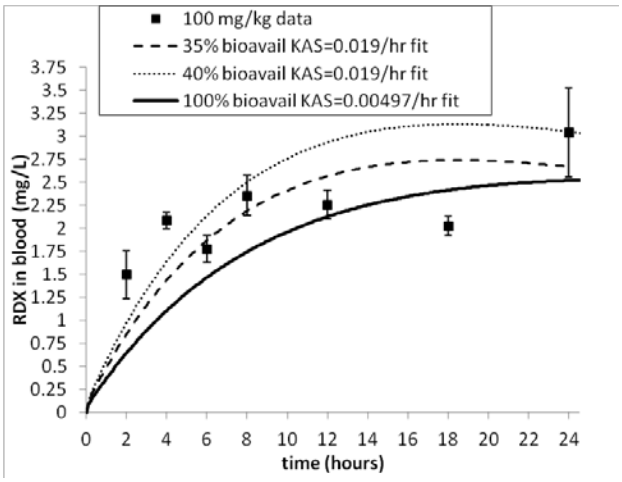


Figure C-5. Effect of varying oral absorption parameters on EPA rat model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations following oral exposure to coarse-grain RDX.[a]

[a]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); see 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.

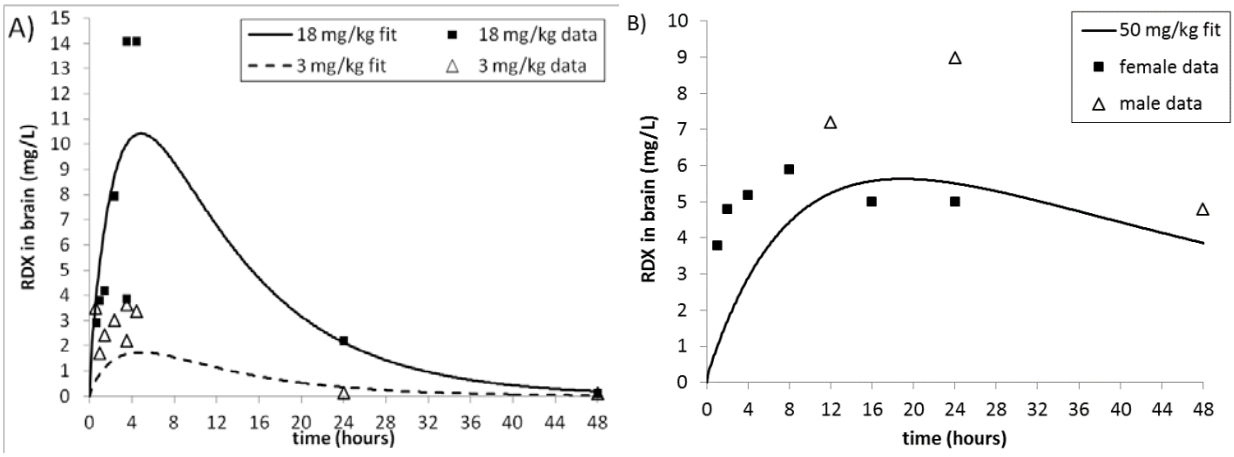


Figure C-6. EPA rat model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) brain tissue concentrations following oral exposure to RDX.[a]

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

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. (2009)	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 and Human PBPK Model.

Additional Hexahydro-1,3,5-trinitro-1,3,5-triazine (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) (see 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) (see 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.

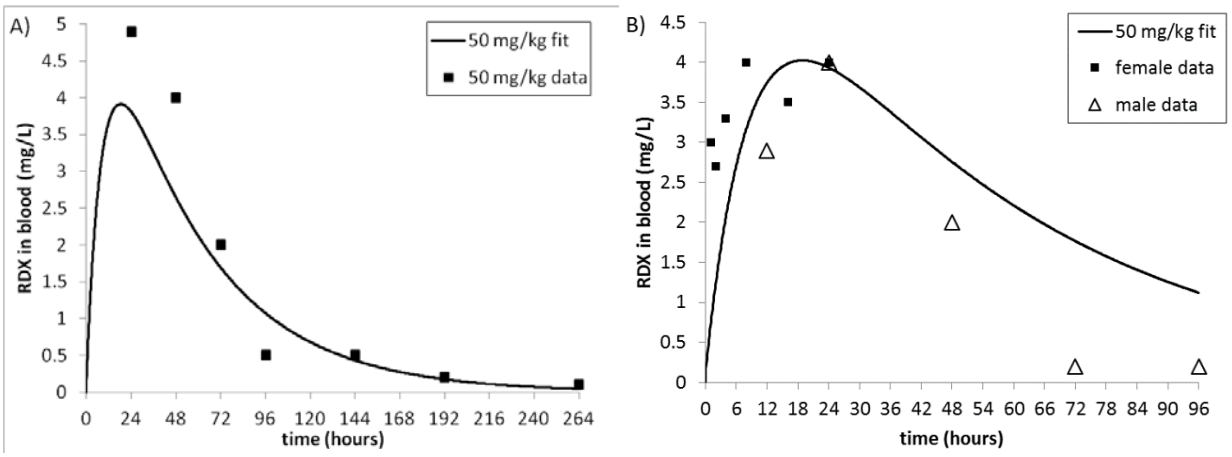


Figure C-7. EPA rat model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations following oral exposure to fine-grain RDX in a saline slurry.[a]

[a]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.

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 in which 20 mg/kg RDX was administered in saline slurry and a drinking water study in which 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 (see 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 (see Figure C-9, symbols). This is counter to the expectation that higher doses cause higher blood levels and is discussed further below.

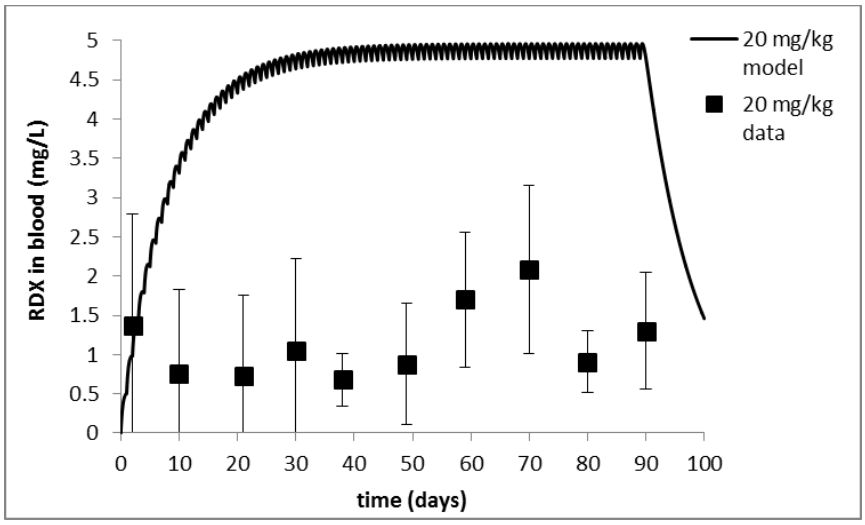


Figure C-8. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic gavage study.[a]

[a]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-grain with an oral absorption rate constant KAS = 0.00497/hour.

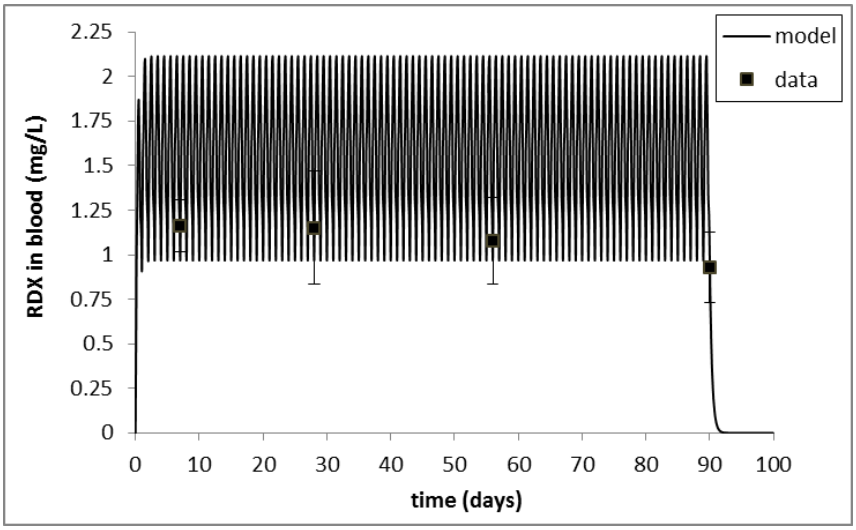


Figure C-9. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic drinking water study.[a]

[a]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).

EPA's modified PBPK model was set up to simulate drinking water exposures with a noncontinuous sipping pattern based on Spiteri (1982), who 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 (see Figure C-8), while for the subchronic drinking water study, the model fit the data reasonably well (see 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 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 Figure C-10. The model predictions for the Özhan et al. (2003)

[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.

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.

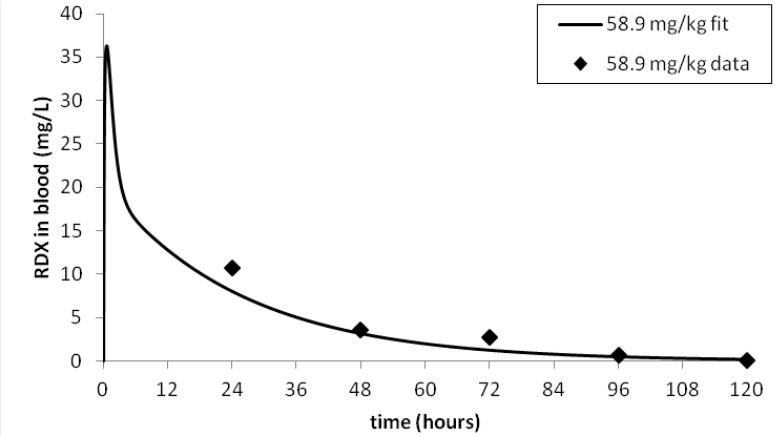


Figure C-10. EPA human model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations resulting from an accidental ingestion of RDX by a 14.5-kg boy (Woody et al., 1986).[a]

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

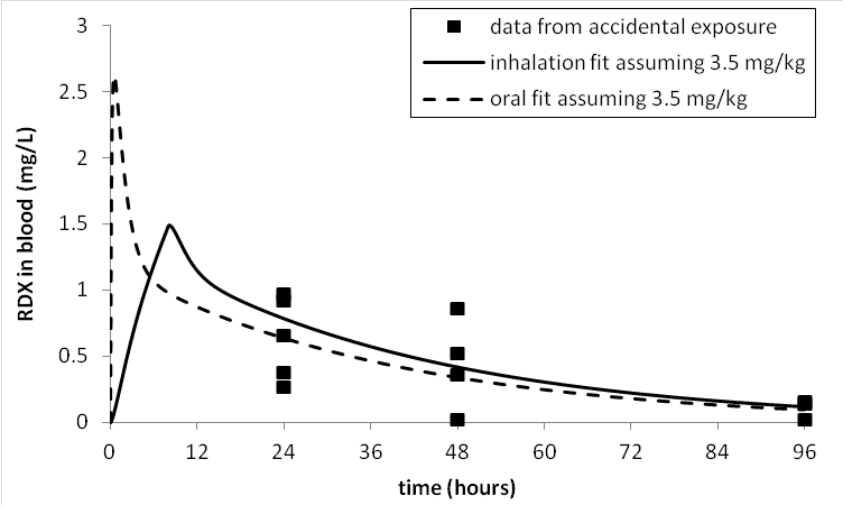


Figure C-11. EPA human model predictions fitted to observed hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) blood concentrations resulting from accidental exposure to adults assumed to be 70 kg (Özhan et al., 2003).

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.

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 Physiologically Based Pharmacokinetic (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 cardiac output (KQC) and fractional blood flow to liver (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 PL in addition to the same parameters (KfC, fractional tissue volume of liver [KVL], and body weight [BW]) found for the blood AUC.

Table C-7. Sensitivity coefficients for rat and human hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) physiologically based pharmacokinetic (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

KQB = fractional blood flow to brain; KQF = fractional blood flow to fat; KVB = fractional tissue volume of brain; KVF = fractional tissue volume of fat; KVV = fractional tissue volume of blood volume.
Parameters with sensitivity coefficients <0.1 in absolute value are considered not sensitive, and are listed below:

- KQC
- Fractional blood flow to all tissues (KQL; KQF; KQS; KQB)
- Fractional tissue volume of KVF, KVB, and KVV
- Blood partition coefficients to all tissues (PL; PF; rapidly PR; PS; 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 (see Section C.1.1), estimates of the bioavailability of RDX range from 50 to 87% or greater and may depend on 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 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 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.

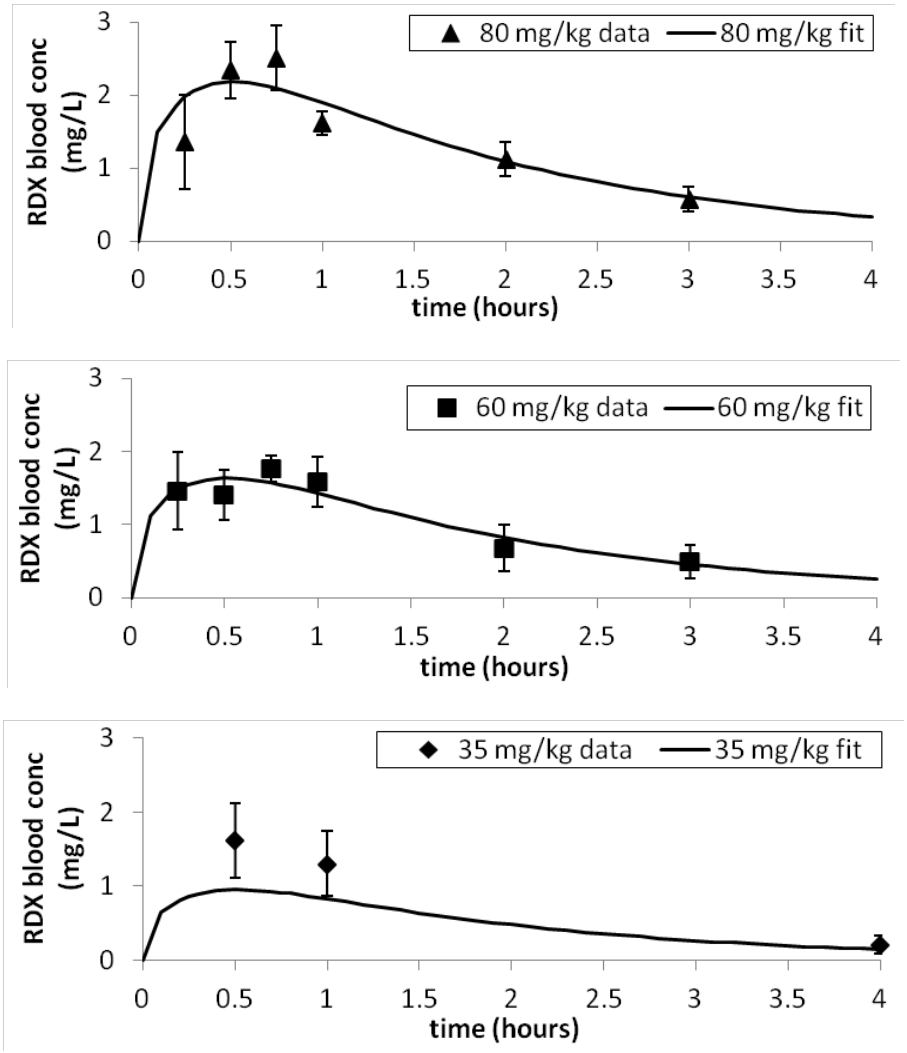


Figure C-12. Comparison of EPA mouse physiologically based pharmacokinetic (PBPK) model predictions with data from oral exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) dissolved in water.[a]

[a]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).

The mouse RDX blood concentrations reported by Sweeney et al. (2012b), as shown in Figure C-12 were evaluated with a noncompartmental 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:

$$AUC_{total} = \Sigma(\Delta\text{blood concentrations})\ \Delta t/2 + \text{blood concentration at last time point}/K_{el} \quad \text{(C-1)}$$

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 (see 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 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 noncompartmental 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. Tissue-specific dose metrics for kidney, bladder, and prostate were not available in the PBPK model. The PBPK model estimates RDX concentrations in the brain and these were considered as tissue-specific dose metrics for

neurotoxicity. The brain RDX concentrations were not used because the brain concentration data are limited and the data are only moderately well fit. Based on the limited number of observations of brain concentrations in toxicokinetic studies, the brain concentrations correlate with plasma concentrations. The plasma concentrations were calibrated by fitting to multiple data set and predictions from the rat model compared well with data not used in model calibration, supporting the use of plasma concentrations.

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 benchmark dose lower confidence limit (BMDL) in rats by the ratio of these slopes as follows:

- For a gavage dose in rats converted to a human drinking water dose (i.e., applied to a study that used gavage dosing, specifically Crouse et al. (2006) and Cholakis et al. (1980)):
  - The ratio for AUC was 6.800 / 13.95 = 0.487
  - The ratio for C_{max} was 0.4718 / 0.7316 = 0.645
- For a continuous dose in rats converted to a human drinking water dose (i.e., applied to a study that used dietary dosing, specifically Levine et al. (1983)):
  - The ratio for AUC was 6.800 / 13.95 = 0.487
  - The ratio 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 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 AUC and 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 as follows:

- For a gavage dose in mice converted to a human drinking water dose (i.e., applied to a study that used gavage dosing):
  - The ratio for AUC was 0.0656 / 13.95 = 0.0047
  - The ratio for C_{max} was 0.0273 / 0.7316 = 0.373, respectively.
- For a continuous dose in mice converted to a human drinking water dose (i.e., applied to a study that used dietary dosing):
  - The ratio for AUC was 0.0656 / 13.95 = 0.0047
  - The ratio for C_{max} was 0.0081 / 0.7316 = 0.011

Summary of Confidence in Physiologically Based Pharmacokinetic (PBPK) Models for hexahydro-1,3,5-trinitro-1,3,5-triazine (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 time point 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 nondetects, 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 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) Physiologically Based Pharmacokinetic (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.

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 1.2.7. 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 (see 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.

Table C-8. Summary of case reports of exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (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)

Table C-8. Summary of case reports of exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

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, semicomatose, 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 was 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 days), 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)

Table C-8. Summary of case reports of exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Ketel and Hughes (1972) 18 males (December 1968–December 1969) Wartime, Vietnam	Inhalation while cooking with C-4 and possible ingestion	Central nervous system 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
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 × 109/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	

Table C-8. Summary of case reports of exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
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	
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 alanine aminotransferase; kidney damage; plasma RDX levels 3 hrs after ingestion ranged from 268 to 969 pg/mL	

Table C-8. Summary of case reports of exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Davies et al. (2007) 17 males Nonwartime	Ingestion of an unknown quantity of 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	

AST = aspartate aminotransferase ; BUN = blood urea nitrogen; CSF = cerebrospinal fluid; EEG = electroencephalogram; WBC = white blood cell.

Table C-9. Occupational epidemiologic studies of hexahydro-1,3,5-trinitro-1,3,5-triazine (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)

Table C-9. Occupational epidemiologic studies of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX): summary of methodologic features (continued)

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
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 exposed groups (nondetected, >0.01 mg/m^3) based on subject's industrial hygiene monitoring results.	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)

Table C-9. Occupational epidemiologic studies of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX): summary of methodologic features (continued)

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
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 (2.0×10^9/L), low platelet count ($<150 \times 10^9$/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)

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

[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.

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

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr (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-mo interim sacrifice)	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	Mortality at 11 wk (*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 mos (*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 yr *(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 mos 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 yr (mortality incidence also provided for mice through wk 13, and from the report of the 6-mo scheduled sacrifice)	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Mortality at 13 wk (*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-mos 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 yr (*incidence*)					
	M	17/55	19/55	30/55*	26/55	51/55*
	F	12/55	10/55	13/55	14/55	27/55*

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

Reference and study design	Results					
Cholakis et al. (1980) Mice, B6C3F$_1$, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 40, 60, or 80 mg/kg-d for 2 wk 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 wk	**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 wk	**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 wk premating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wk 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 wk	**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

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

Reference and study design	Results						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[d] 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 wk	**Doses**	**0**	**10**	**30**	**100**	**300**	**600**
	Mortality (*incidence*)[e]						
	M	0/30	0/10	0/10	8/10	10/10	10/10
	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 wk	**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 Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**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 wk	**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 wk	**Doses**	**0**	**50**				
	Mortality (*incidence*)						
	F	0/5	1/7				

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

Reference and study design	Results						
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).						
Cholakis et al. (1980) Rabbits, New Zealand White, 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

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

Reference and study design	Results				
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 2, 6, or 20 mg/kg-d Gavage GDs 6–15	**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		

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average.
*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-d died by Week 3 of the study; animals receiving 600 mg/kg-d 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).

Following chronic dietary exposure, an increased rate of mortality in male F344 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). Mice were less sensitive than rats with respect to mortality following a similar 2-year exposure to RDX. 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., 1984b). 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., 1984b; 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

[4]Deaths in high-dose (40 mg/kg-day) male rats were reported beginning around month 3 to 4 [estimated from Volume 1, Figure 1 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 [see Table 4 in Levine et al. (1983)].

8–15 mg/kg-day RDX, preterm deaths occurred in male rats as early as Days 26–78 and in female rats as early as Days 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 also 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. (1984b) 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 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 those 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).

C.3.2. Other Noncancer Effects

There are some reports of RDX inducing systemic effects in several organs/systems, including the eyes and the cardiovascular, musculoskeletal, immune, GI, hematological, and male reproductive systems, and body weight. However, there is less evidence for these effects compared to organ systems described in Section 1.2. Overall, at the present time, there is inadequate information to identify 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 to C-18, 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 (see Table C-11). 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 study 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., 1984b). A statistically significant increase in the incidence of cataracts in male mice was initially noted by Lish et al. (1984b), 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. There is insufficient information to assess ocular toxicity following exposure to RDX.

Table C-11. Evidence pertaining to ocular effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	Cataracts; 103 wk (*incidence*)[a]					
	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 mos 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 yr	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Cataracts; 103 wk (*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 wk	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 wk	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,[b] 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wk	No ocular effects were observed (ophthalmoscopic examination was performed at the end of exposure).					

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.
*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]Incidence counts exclude individuals from which blood was obtained via the orbital sinus.
[b]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).

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 (see Table C-12). 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., 1984b; 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. There is insufficient information to assess cardiovascular effects following exposure to RDX.

Table C-12. Evidence pertaining to cardiovascular effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	Absolute heart weight; 104 wk (*percent change compared to control*)					
	M	0%	4%	4%	5%	7%
	F	0%	1%	5%	2%	−5%
	Relative heart-to-body weight; 104 wk (*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 yr	**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 wk (*percent change compared to control*)					
	M	0%	−6%	−2%	−5%	
	F	0%	13%	3%	15%	
	Relative heart-to-body weight; 104 wk (*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 mos 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 yr	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Absolute heart weight; 104 wk (*percent change compared to control*)					
	M	0%	3%	−2%	−2%	1%
	F	0%	−1%	0%	−4%	−3%
	Relative heart-to-body weight; 104 wk (*percent change compared to control*)					
	M	0%	2%	6%	0%	22%
	F	0%	−2%	3%	−1%	15%

Table C-12. Evidence pertaining to cardiovascular effects in animals (continued)

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 wk	**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 wk followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wk	**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 wk	**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*)						

Table C-12. Evidence pertaining to cardiovascular effects in animals (continued)

Reference and study design	Results						
	M	0%	—	—	—	−4%	−10%*
	F	0%	—	—	—	−5%	−11%*
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wk premating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wk 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 wk	**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)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wk	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%	—	—

Table C-12. Evidence pertaining to cardiovascular effects in animals (continued)

Reference and study design	Results						
	Relative heart-to-body weight (*percent change compared to control*)						
	M	0%	2%	−4%	3%	—	—
	F	0%	−2%	0%	−3%	—	—
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 wk	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 Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**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 wk	**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%		

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average.
*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[c]The species of monkey used in this study was inconsistently reported in the study as either cynomolgus (in the Methods section) or rhesus (in the Summary).
Note: A dash ("—") indicates that the study authors did not measure or report a value for that dose group.

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), creatine phosphokinase, and myoglobinuria (Testud et al., 2006; Küçükardali et al., 2003; Hett and Fichtner, 2002; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968) (see Appendix C, Section C.2). Histological evaluations of musculature or skeletal tissue did not reveal any alterations in mice (Lish et al., 1984b) 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). In summary, based on a limited number of case reports of muscle involvement involving acute exposure to RDX, there is insufficient information to assess musculoskeletal effects following exposure to RDX.

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 white blood cell (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 (see Table C-13), 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 that evaluated structural measures of immunotoxicity (including 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., 1984b) provide no evidence of immunotoxicity associated with oral (dietary) exposure to RDX. None of the available studies included evaluation of more sensitive measures of functional immune system changes that would be more likely to detect immunosuppression, unintended immune stimulation, autoimmunity, or dysregulated inflammation.

One special case of an immune response potentially relevant to RDX is neuroinflammation that may result from recurrent seizures. Neuroinflammation is a key characteristic of most neurological conditions, including seizure and epilepsy (Eyo et al., 2017; Dey et al., 2016). Hypothetically, RDX-induced seizures could indirectly trigger acute immune and inflammatory responses from microglia within the brain; additionally, chronic neuroinflammation may result from recurrent seizures. While this hypothesized relationship between an inflammatory response and seizures may be relevant to the convulsant effects of RDX, the relationship to the less severe manifestations of RDX neurotoxicity is unclear. Further, there is no available information on neuroinflammatory responses after exposure to RDX.

In summary, evidence for immunotoxicity associated with RDX exposure is limited to findings from several case reports indicating increased WBC counts in Vietnam War troops who ingested or inhaled RDX and changes in WBC counts in rats in two studies (Levine et al., 1981a, b; Hart, 1976) that were not consistent in direction or necessarily across sexes, and were not supported by dose-dependent patterns of response across the RDX database. Finally, no dose-related immune effects were observed in a 90-day rat study that evaluated structural, but not functional, immunotoxicity endpoints (Crouse et al., 2006). Therefore, there is insufficient information to assess immunotoxicity following exposure to RDX.

Table C-13. Evidence pertaining to immune effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	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 wk (*percent change compared to control*)					
	M	0%	−13%	−8%	−16%	−30%
	F	0%	12%	39%*	28%	0%
	Absolute spleen weight; 105 wk (*percent change compared to control*)					
	M	0%	24%	31%	−10%	−28%
	F	0%	4%	15%	−17%	16%
	Relative spleen weight; 105 wk (*percent change compared to control*)					
	M	0%	26%	32%	−11%	−21%
	F	0%	4%	15%	−17%	44%
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 yr	**Doses**	**0**	**1.0**	**3.1**	**10**	
	WBC count; 104 wk (*percent change compared to control*)					
	M	0%	−13%	−22%*	−34%*	
	F	0%	5%	−32%*	−12%	
	Absolute spleen weight; 104 wk (*percent change compared to control*)					
	M	0%	−11%	−16%	−4%	
	F	0%	58%	8%	37%	
	Relative spleen weight; 104 wk (*percent change compared to control*)					
	M	0%	−11%	−14%	1%	
	F	0%	77%	19%	55%	

Table C-13. Evidence pertaining to immune effects in animals (continued)

Reference and study design	Results					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 yr	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 wk (*percent change compared to control*)					
	M	0%	−11%	103%[a]	184%[a]	15%
	F	0%	7%	12%	354%[a]	251%[a]
	Absolute spleen weight; 105 wk (*percent change compared to control*)					
	M	0%	5%	−10%	−32%	−49%
	F	0%	−28%	−44%	−35%	17%
	Relative spleen weight; 105 wk (*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 wk	**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%
Experiment 2: 0, 40, 60, 80 mg/kg-d for 2 wk 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 wk	**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%

Table C-13. Evidence pertaining to immune effects in animals (continued)

Reference and study design	Results						
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 wk	**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 wk premating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wk after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	No immune effects were observed upon routine histopathology evaluation.						

Table C-13. Evidence pertaining to immune effects in animals (continued)

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 wk	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)[c] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wk	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%	—	—

Table C-13. Evidence pertaining to immune effects in animals (continued)

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 wk	**Doses**	**0**	**15**	**25**	**50**
	WBC count (*percent change compared to control*)				
	M	0%	−30%	7%	−6%
Hart (1974) Dogs, beagle, 3/sex/group Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**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,[d] 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wk	**Doses**	**0**	**0.1**	**1**	**10**
	WBC count (*percent change compared to control*)				
	M	0%	−32%	0%	−3%
	F	0%	−38%	−1%	−41%

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average.
*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]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.
[b]Doses were calculated by the study authors.
[c]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[d]The species of monkey used in this study was inconsistently reported in the study as either cynomolgus (in the Methods section) or rhesus (in the Summary).

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

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).

Additionally, Küçükardali et al. (2003) reported several cases of erosive gastroduodenitis in individuals acutely exposed to RDX, suggesting severe irritation of the GI tract by direct contact with RDX. In animal studies (see Table C-14), 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., 1984b; 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. There is some evidence that direct contact with RDX in the GI tract may cause erosive gastroduodenitis in poisoning victims; however, histopathological changes were not generally reported in experimental animals exposed to RDX in the diet. There is insufficient information to assess gastrointestinal toxicity following exposure to RDX.

Table C-14. Evidence pertaining to gastrointestinal effects in animals

Reference and study design	Results
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	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 mos 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 yr	No GI tract effects were observed as clinical signs or on gross pathology or histopathology examination.
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wk	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 wk	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,[a] 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wk	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.

Table C-14. Evidence pertaining to gastrointestinal effects in animals (continued)

Reference and study design	Results
Hart (1974) Dogs, beagle, 3/sex/group Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	Some nausea and vomiting were reported (incidences and affected dose groups were not reported).

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.
[a]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).

Hematological Effects

Elevated prevalence odds ratios (ORs) for hematological abnormalities (i.e., neutropenia, low platelet count, or macrocytosis; see Table C-15 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-15). 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 coexposures. 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-16). 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., 1984b;

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 a case-control study and a cross-sectional study were inconsistent. Incidences of anemia observed in human case reports may reflect coexposures to trinitrotoluene (TNT); anemia has been reported in F344 rats exposed to TNT, but not RDX (Levine et al., 1990; Levine et al., 1981a, b). The small number of cases in the case-control study and exposed individuals in the cross-sectional study contribute to the difficulty in interpreting the results across studies (see Table C-15). In general, animal studies of chronic and subchronic durations showed no consistent, dose-related pattern of increase or decrease in hematological parameters. There is insufficient information to assess hematological toxicity following exposure to RDX.

Table C-15. 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 measures**: Exposure determination based on employee interviews and job title analysis; data included frequency (hrs/d, d/yr), duration (yr), 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 × 109/L), low platelet count (<150 × 109/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 C-15. Evidence pertaining to other noncancer effects (hematological) in humans (continued)

<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>
*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>
*Includes both workers exposed to RDX alone and RDX and HMX.
No differences were statistically significant. Similar results in women.</td></tr>
</table>

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; LOD = limit of detection.

Table C-16. Evidence pertaining to hematological effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	RBC count; 105 wk (*percent change compared to control*)					
	M	0%	−4%	3%	−3%	14%
	F	0%	4%	−7%	5%	3%
	Hemoglobin; 105 wk (*percent change compared to control*)					
	M	0%	−6%	3%	−5%	9%
	F	0%	2%	−7%	3%	1%
	Hematocrit; 105 wk (*percent change compared to control*)					
	M	0%	−4%	3%	−4%	9%
	F	0%	3%	−6%	3%	1%
	Platelets; 105 wk (*percent change compared to control*)					
	M	0%	33%	9%	21%	27%
	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 yr	**Doses**	**0**	**1.0**	**3.1**	**10**	
	RBC count; 104 wk (*percent change compared to control*)					
	M	0%	3%	7%	−2%	
	F	0%	−14%	7%	2%	
	Reticulocyte count; 104 wk (*percent change compared to control*)					
	M	0%	250%	500%*	850%*	
	F	0%	180%*	−40%	20%	
	Hemoglobin; 104 wk (*percent change compared to control*)					
	M	0%	3%	4%	0%	
	F	0%	−1%	1%	−2%	

Table C-16. Evidence pertaining to hematological effects in animals (continued)

Reference and study design	Results					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 yr	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Hemoglobin levels; 105 wk (*percent change compared to control*)					
	M	0%	6%	6%	3%	−13%
	F	0%	−5%	1%	−9%	−14%
	RBC count; 105 wk (*percent change compared to control*)					
	M	0%	5%	2%	−1%	−9%
	F	0%	−2%	2%	−9%	−13%
	Platelet count; 105 wk (*percent change compared to control*)					
	M	0%	6%	−4%	−10%	−7%
	F	0%	14%	−4%	5%	22%
	Hematocrit; 105 wk (*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 wk followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wk	**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%	
	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%	

Table C-16. Evidence pertaining to hematological effects in animals (continued)

Reference and study design	Results						
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 wk	**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 wk	**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%
	Hematocrit (*percent change compared to control*)						
	M	0%	2%	−5%	0%	−1%	−4%
	F	0%	3%	4%	0%	4%	−2%

Table C-16. Evidence pertaining to hematological effects in animals (continued)

Reference and study design	Results						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wk	Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all 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 wk	**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 Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**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%		
	Hemoglobin (*percent change compared to control*)						

Table C-16. Evidence pertaining to hematological effects in animals (continued)

Reference and study design	Results				
	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 wk	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%

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average.
*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[c]The species of monkey used in this study was inconsistently reported in the study as either cynomolgus (in the Methods section) or rhesus (in the Summary).
Note: A dash ("—") indicates that the study authors did not measure or report a value for that dose group.

Reproductive Effects

Female Reproductive Effects

Reproductive function in rats was assessed in a two-generation study by Cholakis et al. (1980). No specific effects on reproductive function were observed in F0 and F1 CD rats exposed to ≤16 mg/kg-day RDX. A reduction in the number of pregnancies was reported following mating of the F0 generation at the highest dose tested (50 mg/kg-day) (89% pregnancies in controls versus 69% at 50 mg/kg-day); exposure at this dose also resulted in decreased food consumption relative

to controls (14 and 17% less in females and males, respectively, at Week 13 of exposure), decreased body weight relative to controls (8 and 15% less in females and males, respectively, at Week 13), and mortality in 9% of male rats and 27% of female rats. The study authors considered the effects on reproductive function likely due to the general toxicity of RDX rather than a direct effect of RDX on reproduction. In a dominant lethal mutation study that used the same F0 males from the two-generation reproductive toxicity study but mated a second time to untreated female rats, pregnancy rates were again lower in untreated females mated to high-dose (50 mg/kg-day) males (97% in controls versus 79% in females mated with high-dose males). The study authors also attributed this effect to adverse effects of treatment on the general health of the high-dose males (Cholakis et al., 1980).

The limited investigation of reproductive function in RDX-exposed rats by a single investigator (Cholakis et al., 1980) provides insufficient information to assess female reproductive toxicity following exposure to RDX.

Male Reproductive Effects

Evidence of male reproductive toxicity comes largely from the finding of increased incidence of testicular degeneration (10−11%) in male B6C3F$_1$ mice exposed to ≥35 mg/kg-day RDX for 2 years in the diet compared to concurrent controls (0%) (Lish et al., 1984b) (see Table C-17). The biological significance of this finding is unclear. As noted by the Science Advisory Board (SAB) in its review of external review draft of the RDX assessment (SAB, 2017), no histopathological changes were observed in this study in animals sacrificed at 6 or 12 months, durations longer than the 1.4-month duration of spermatogenesis in mice. Significant decreases in testes weight would generally be expected where there is appreciable testicular degradation; however, reductions in absolute testicular weight in male mice in Lish et al. (1984b) were small (≤6% compared to controls) and not dose related. The SAB also noted that, in general, the validity of 2-year chronic toxicity studies for evaluating male reproductive toxicity is questionable because of the loss of testicular function that occurs with aging rodents (SAB, 2017). In 2-year old mice, studies have shown reductions in sperm counts and motility, hormone levels, and numbers of spermatogonial stem cells, loss of functional ability of spermatogonial stem cells, and failure of the somatic environment to support spermatogonial differentiation (Zhang et al., 2006; Gosden et al., 1982; Suzuki and Withers, 1978; Bronson and Desjardins, 1977). These age-related changes in male reproductive organs confound the interpretation of effects of a potential reproductive toxicant.

The evidence for testicular degeneration in mice suggested by Lish et al. (1984b) was generally not supported by other studies. In particular, Cholakis et al. (1980) found no histopathological changes in the testes in the same strain of mice (B6C3F$_1$) exposed to RDX for 3 months at a dose of 320 mg/kg-day. This subchronic duration would have allowed for two complete rounds of spermatogenic cell differentiation and therefore sufficient time for an effect of RDX on male reproductive organs to have been detected. No dose-related histopathological

changes in the testes were identified in the majority of studies in rats (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a, b; Hart, 1976) or in dogs (Hart, 1974). In a 2-year bioassay in F344 rats (Levine et al., 1983), an increased incidence of germ cell degeneration (40%) was observed in 12-month interim-sacrifice rats exposed to 40 mg/kg-day compared with controls (0%), as well as a 14% decrease in testis weight. Because RDX caused 30–40% excess mortality by 12 months at this dose, testicular effects could have been secondary to general toxicity. Histopathological findings obtained at 2 years were not meaningful because almost all male rats (including controls) developed testicular masses (interstitial cell tumors)—a finding typical for rats of this strain.

Changes in testes weight were not consistent with effects of RDX on the male reproductive system. Across studies that measured this endpoint, changes in testes weight were generally small (≤10% compared to control), not dose related, and directionally inconsistent, with testes weights both increased and decreased relative to the control (see Table C-17).

In light of inconsistent findings of histopathology changes in the testes across studies, questions about the validity of 2-year chronic toxicity studies for evaluating male reproductive toxicity, and lack of supporting evidence from organ-weight measurements, there is insufficient information to assess male reproductive toxicity following exposure to RDX.

Table C-17. Evidence pertaining to male reproductive effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 Week 11 due to excessive mortality) Diet 2 yr	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	Testicular degeneration *(incidence)*					
		0/63	2/60	2/62	6/59	3/27[a]
	Absolute testes weight; Week 105 *(percent change compared to control)*					
		0%	−6%	0%	−2%	−6%
Hart (1976) Rats, Sprague-Dawley, 100/sex/dose Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yr	**Doses**	**0**	**1.0**	**3.1**	**10**	
	Absolute testes (with epididymis) weight; Week 104					
		0%	−2%	2%	5%	
	Testes were examined microscopically in control and 10 mg/kg-d groups; no degeneration or other treatment-related effects were observed.					
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos 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 yr	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Testes, germ cell degeneration; 12 mos *(incidence)*					
		0/10	0/10	0/10	0/10	4/10*
	Testes, germ cell degeneration; 24 mos *(incidence)*					
		0/54	0/55	0/52	0/55	0/31
	Absolute gonad weight; 12 mos *(percent change compared to control)*					
		0%	0%	+1%	0%	−14%
	Testes weights were not measured at termination due to testicular masses in nearly all males.					
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 wk	**Doses**	**0**	**10**	**14**	**20**	**28**	**40**
	Absolute testes weight *(percent change compared to control)*						
		0%	—	—	—	−4%	−4%

Table C-17. Evidence pertaining to male reproductive effects in animals (continued)

Reference and study design	Results						
Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wk 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 wk	**Doses**	**0**	**80**	**160**	**320**		
	Absolute testes weight *(percent change compared to control)*						
		0%	4%	−4%	−8%		
	Testes were examined microscopically in control and 320 mg/kg-d groups; no effects were observed.						
Cholakis et al. (1980) Rats, F344, 10/sex/dose 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 wk	**Doses**	**0**	**10**	**14**	**20**	**28**	**40**
	Absolute testes weight *(percent change compared to control)*						
		0%	—	—	—	−2%	0%
	Testes were examined microscopically in control and 40 mg/kg-d groups; no effects were observed.						
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wk	**Doses**	**0**	**4**	**8**	**10**	**12**	**15**
	Absolute testes weight *(percent change compared to control)*						
		0%	−3%	−5%	−4%	−4%	−8%
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[c] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wk	**Doses**	**0**	**10**	**30**	**100**	**300**	**600**
	Testes, germ cell degeneration *(incidence)*						
		0/10	0/10	0/10	0/10	—	—
	Absolute testes weight *(percent change compared to control)*						
		0%	1%	1%	−2%	—	—
	Note: all animals in the 300- and 600-mg/kg-d groups died within the first 3 weeks of the study. Therefore, data for these groups were not reported here.						

Table C-17. Evidence pertaining to male reproductive effects in animals (continued)

Reference and study design	Results				
Hart (1974) Dogs, beagle, 3/sex/dose Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**Doses**	**0**	**0.1**	**1**	**10**
	Absolute testes (with epididymis) weight *(percent change compared to control)*				
		0%	—	—	51%
	Testes were not examined microscopically.				

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; TWA = time-weighted average.
*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]Although the study authors did not observe a statistically significant increase in the incidence of testicular degeneration, they determined that the incidences at the 35 and 175/100 mg/kg-d dose groups were "notable" when compared to concurrent control incidence.
[b]Doses were calculated by the study authors.
[c]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
Note: A dash ("—") indicates that the study authors did not measure or report a value for that dose group.

Body-Weight Effects

Changes in body-weight gain were reported in experimental animal studies involving chronic and subchronic exposure to ingested RDX, generally at doses that were also associated with other RDX-related toxicity. For example, terminal body weights were more than 10% lower than controls in female mice exposed to 100 mg/kg-day Lish et al. (1984b) and in rats exposed to ≥40 mg/kg-day (Levine et al., 1990; Levine et al., 1983; Cholakis et al., 1980). In these studies, RDX doses at which >10% decrements in body weight were observed were also associated with elevated mortality, and in the Levine et al. (1983) study, with severe kidney and urinary bladder toxicity in male rats. For the most part at lower doses, there were no apparent patterns of treatment-related body-weight changes across dose groups or sexes within a study, or across studies. One exception is the 90-day gavage study in rats by Crouse et al. (2006), where a dose-related increase in body-weight gain was observed in female rats exposed to RDX (26% at the highest dose of 15 mg/kg-day). The study authors did not provide an explanation for the body-weight increase.

In summary, available studies provide evidence that RDX exposure affects body weight in mice and rats, but these effects appear to be secondary to effects on other primary targets of RDX toxicity.

Table C-18. Evidence pertaining to body-weight effects in animals

Reference and study design	Results					
Lish et al. (1984b) Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos. 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 Week 11 due to excessive mortality) Diet 2 yr	**Doses**	**0**	**1.5**	**7.0**	**35**	**175/100**
	Final body weight (g) *(mean ± SD)*					
	M	38.0 ± 3.8	36.5 ± 2.8	37.8 ± 3.8	37.1 ± 2.7	36.1 ± 3.3*
	F	41.7 ± 5.1	41.9 ± 5.6	41.6 ± 6.0	41.1 ± 5.5	33.7 ± 4.9*
	Final body weight *(percent change compared to control)*					
	M	0%	−4%	−1%	−2%	−5%
	F	0%	0%	0%	−1%	−19%
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yr	**Doses**	**0**	**1.0**	**3.1**	**10**	
	Final body weight (g) *(mean ± SE)*					
	M	660.6 ± 21	675.0 ± 28	643.0 ± 26	613.8 ± 32	
	F	450.2 ± 23	399.4 ± 14	368.5 ± 17*	408.2 ± 20	
	Final body weight *(percent change compared to control)*					
	M	0%	2%	−3%	−7%	
	F	0%	−11%	−18%	−9%	
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mos. 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 yr	**Doses**	**0**	**0.3**	**1.5**	**8.0**	**40**
	Final body weight (g) *(mean ± SD)*					
	M	409 ± 43	411 ± 55	377 ± 58	397 ± 41	323 ± 50*
	F	303 ± 23	301 ± 25	292 ± 34	291 ± 38	255 ± 18*
	Final body weight *(percent change compared to control)*					
	M	0%	0%	−8%	−3%	−21%
	F	0%	−1%	−4%	−4%	−16%
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 wk followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a] Diet 13 wk	**Doses**	**0**	**80**	**160**	**320**	
	Final body weight (g) *(mean ± SE)*					
	M	26.5 ± 0.4	27.1 ± 0.4	27.1 ± 0.4	27.3 ± 1.1	
	F	26.0 ± 0.4	25.8 ± 0.3	26.3 ± 0.4	27.5 ± 0.4*	
	Final body weight *(percent change compared to control)*					
	M	0%	2%	2%	3%	
	F	0%	−1%	1%	6%	

Table C-18. Evidence pertaining to body-weight effects in animals (continued)

Reference and study design	Results						
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 wk	**Doses**	**0**	**10**	**14**	**20**	**28**	**40**
	Final body weight (g) *(mean ± SE)*						
	M	306.2 ± 3.7	308.4 ± 5.2	308.3 ± 2.7	303.3 ± 10.0	295.7 ± 3.0	280.6 ± 3.3*
	F	178.7 ± 2.6	176.3 ± 3.4	175.6 ± 1.7	175.5 ± 2.2	171.1 ± 3.1	168.8 ± 3.7
	Final body weight *(percent change compared to control)*						
	M	0%	1%	1%	−1%	−3%	−8%
	F	0%	−1%	−2%	−2%	−4%	−6%
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wk premating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wk after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	**Doses**	**0**	**5**	**16**	**50**		
	Final body weight (g) *(mean ± SE)*						
	M (F0)	530 ± 12	534 ± 12	528 ± 9	453 ± 9*		
	F (F0)	285 ± 5	285 ± 5	280 ± 3	262 ± 5*		
	M (F1)	435 ± 10	426 ± 8	408 ± 7	397[ǂ]		
	F (F1)	258 ± 5	255 ± 4	247 ± 4	233[ǂ]*		
	[ǂ] Mean of four males from one litter or two females from one litter.						
	Final body weight *(percent change compared to control)*						
	M (F0)	0%	1%	0%	−15%		
	F (F0)	0%	0%	−2%	−8%		
	M (F1)	0%	−2%	−6%	−9%		
	F (F1)	0%	−1%	−4%	−10%		
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wk	**Doses**	**0**	**4**	**8**	**10**	**12**	**15**
	Final body weight (g) *(mean ± SD)*						
	M	317.5 ± 14.1	299.1 ± 26.8	289.2 ± 24.5*	308 ± 29.3	321.6 ± 19.3	325.4 ± 15.2
	F	175.6 ± 7.4	174.7 ± 7.1	190.6 ± 23.5	200.9 ± 17.5*	207.8 ± 12.1*	220.8 ± 20.7*
	Final body weight *(percent change compared to control)*						
	M	0%	−6%	−9%	−3%	1%	2%
	F	0%	−1%	9%	14%	18%	26%

Table C-18. Evidence pertaining to body-weight effects in animals (continued)

Reference and study design	Results						
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)[b] Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wk	**Doses**	**0**	**10**	**30**	**100**	**300**	**600**
	Final body weight (g) *(mean ± SD)*						
	M	301.6 ± 23.2	292.1 ± 23.9	276.1 ± 25.0*	251.0*	—	—
	F	170.7 ± 12.1	172.2 ± 6.9	170.0 ± 10.7	173.2 ± 7.9	—	—
	Final body weight *(percent change compared to control)*						
	M	0%	−3%	−8%	−17%	—	—
	F	0%	1%	0%	1%	—	—
Hart (1974) Dogs, beagle, 3/sex/group Premix with ground dog chow containing 20 mg RDX/g-chow, 60 g of dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wk	**Doses**	**0**	**0.1**	**1**	**10**		
	Terminal body weight (kg) *(mean)*						
	M	8.2	8.6	8.0	10.5		
	F	8.1	7.9	8.5	8.1		
	Final body weight *(percent change compared to control)*						
	M	0%	5%	−2%	28%		
	F	0%	−2%	5%	0%		
Martin and Hart (1974) Monkeys, cynomolgus or rhesus,[c] 3/sex/group Purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wk	**Doses**	**0**	**0.1**	**1**	**10**		
	Final body weight (kg) *(mean)*						
	M	3.8	3.7	3.7	3.8		
	F	2.6	2.7	2.8	2.6		
	Final body weight *(percent change compared to control)*						
	M	0%	−3%	−3%	0%		
	F	0%	4%	8%	0%		

HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; SD = standard deviation; SE = standard error; TWA = time-weighted average.
*Significantly different than control ($p \leq 0.05$).
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[c]The species of monkey used in this study was inconsistently reported in the study as either cynomolgus (in the Methods section) or rhesus (in the Summary).
Note: A dash ("—") indicates that the study authors did not measure or report a value for that dose group.

C.3.3. Genotoxicity

Hexahydro-1,3,5-trinitro-1,3,5-triazine (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-19.

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 *Escherichia coli* with and without metabolic activation (Neuwoehner et al., 2007). Similarly, RDX did not induce forward mutations (hypoxanthine-guanine phosphoribosyltransferase 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 fischeri* 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-19. Summary of in vitro studies of the genotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (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)

Table C-19. Summary of in vitro studies of the genotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Reverse mutation (*umu* test)	*Salmonella choleraesuis* subsp. *choleraesuis* (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)

Table C-19. Summary of in vitro studies of the genotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Recombination induction	*S. cerevisiae* D3	30.8 µg/mL	–	–	Metabolic activation with S9	Cotruvo et al. (1977)
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)

DNA = deoxyribonucleic acid.
[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 deoxyribonucleic acid (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 genotoxicity studies are available; these are summarized in Table C-20. 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 hexahydro-1,3,5-trinitro-1,3,5-triazine (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). Minipigs 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-21.

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).

Table C-20. Summary of in vivo studies of the genotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (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)

Table C-21. Summary of in vitro and in vivo studies of the genotoxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (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. Two in vitro studies using more sensitive assays and conditions for detecting mutagenicity found positive results for RDX. 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 details on evaluating dose-response and determining 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, 2012).

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 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)
Convulsions Crouse et al. (2006)[a]	F344 rat/female	0 4 8 10 12 15	0/10 (0%) 0/10 (0%) 2/10 (20%) 3/10 (30%) 5/10 (50%) 5/10 (50%)
	F344 rat/male	0 4 8 10 12 15	0/10 (0%) 0/10 (0%) 1/10 (10%) 3/10 (30%) 8/10 (80%) 7/10 (70%)
	F344 rat/male and female, 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)	F344 rat/female (gestational exposure)	0 0.2 2 20	0/24 (0%) 0/24 (0%) 1/24 (4%) 18/24 (75%)
Urinary bladder: hemorrhagic/suppurative cystitis Levine et al. (1983)	F344 rat/male	0 0.3 1.5 8 40	0/54 (0%) 2/55 (4%) 1/52 (2%) 1/51 (2%) 18/31 (58%)
Prostate suppurative inflammation Levine et al. (1983)	F344 rat/male	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 Wald χ^2 test ($p \geq 0.05$). 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 seizures 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 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total (%)[a]
Convulsion or mortality Johnson (2015)	F344 rat/female	0	0/10 (0%)
		4	0/10 (0%)
		8	3/10 (30%)
		10	5/10 (50%)
		12	9/10 (90%)
		15	8/10 (80%)
	F344 rat/male	0	0/10 (0%)
		4	0/10 (0%)
		8	2/10 (20%)
		10	4/10 (40%)
		12	8/10 (80%)
		15	7/10 (70%)
	F344 rat/male and female, 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 logistic regression-based test ($p \geq 0.05$). 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 χ^2 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 estimating the benchmark dose (BMD). This model selection was conducted in two stages: first, from among only the multistage models to determine a representative multistage model, and second, from among the representative multistage model and the nonmultistage models. In each stage, the benchmark dose lower confidence limit (BMDL) estimates (95% lower confidence limit

[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.

on the BMD, as estimated by the profile likelihood method) and Akaike's information criterion (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.

D.1.2. Modeling Results

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

Nervous System Effects

Table D-3 (and Figure D-1) presents the BMD modeling results for incidence of convulsions for 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-4 (and Figure D-2) presents 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-5 (and Figure D-3) 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 male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage for 90 days (Crouse 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			
Gamma	0.484	101.79	5.78	3.80	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 nonmultistage models, the Multistage 3° model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.231	104.55	5.13	3.49	
LogLogistic	0.512	101.66	5.74	3.85	
Probit	0.291	103.61	5.29	3.43	
LogProbit	0.557	101.25	6.01	4.20	
Weibull	0.369	102.91	5.11	3.18	
Multistage 4°[b]	0.502	100.91	5.19	2.65	
Multistage 3°	**0.502**	**100.91**	**5.19**	**2.66**	
Multistage 2°	0.364	103.03	3.47	2.20	
Quantal-Linear	0.0369	111.56	1.13	0.860	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-d were 0, −0.69, −0.25, −0.06, 1.62, −1.08, respectively. The BMD_{10} and $BMDL_{10}$ values for the selected model were 6.60 and 4.59 mg/kg-d, respectively; the BMD_{01} and $BMDL_{01}$ values for the selected model were 3.02 and 0.569 mg/kg-d, 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.

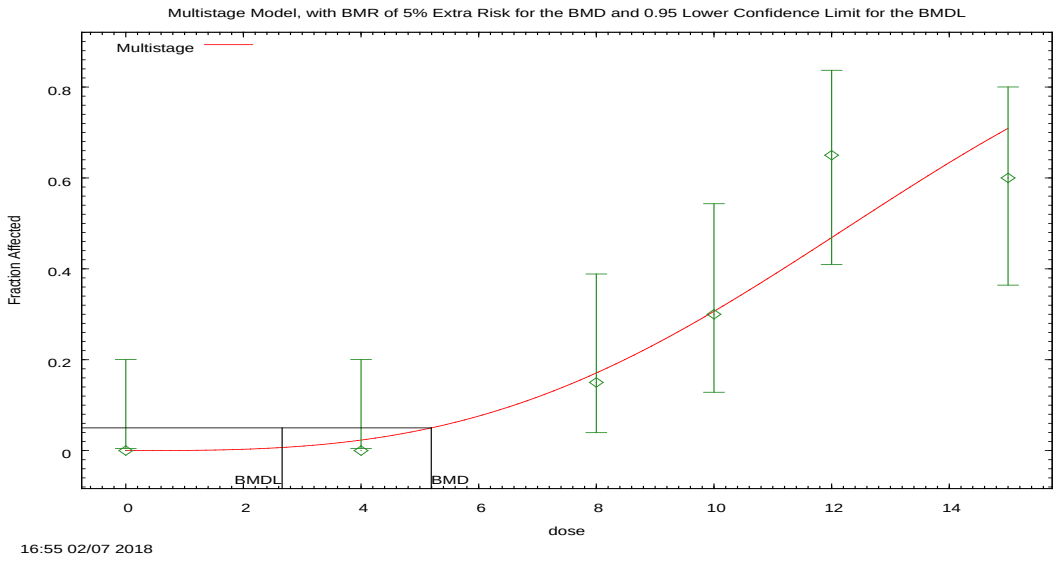


Figure D-1. Plot of incidence rate by dose, with the fitted curve of the Multistage 3° model, for convulsions in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage for 90 days (Crouse et al., 2006). BMR = 5% ER; dose shown in mg/kg-day.

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 = 5% Extra risk

BMD = 5.19399

BMDL at the 95% confidence level = 2.65815

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	−47.08	6			
Fitted model	−49.46	1	4.75213	5	0.45
Reduced model	−71.53	1	48.8965	5	<.0001

d.f. = degrees of freedom.

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	−0.69
8	0.1709	3.418	3	20	−0.25
10	0.3065	6.131	6	20	−0.06
12	0.4688	9.375	13	20	1.62
15	0.7093	14.186	12	20	−1.08

$\chi^2 = 4.34$ degrees of freedom (d.f.) = 5 p-value = 0.5021

Table D-4. Model predictions for convulsions in female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage on gestational days (GDs) 6–19 (Cholakis et al., 1980); 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			
Gamma	0.989	42.003	2.31	0.759	Of the multistage models, the Quantal-Linear model was selected based on lowest AIC. From among the Quantal-Linear and nonmultistage models, the Quantal-Linear model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.526	43.556	6.53	3.90	
LogLogistic	0.991	41.996	2.27	0.823	
Probit	0.577	43.348	5.41	3.34	
LogProbit	1.000	41.963	2.18	0.902	
Weibull	0.983	42.026	2.36	0.756	
Multistage 3°[b]	0.960	42.113	2.51	0.747	
Multistage 2°[b]	**0.960**	**42.113**	**2.51**	**0.747**	
Quantal-Linear	**0.669**	**42.077**	**0.915**	**0.628**	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-d 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-d, respectively; the BMD_{01} and $BMDL_{01}$ values for the selected model were 0.179 and 0.123 mg/kg-d, respectively.

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

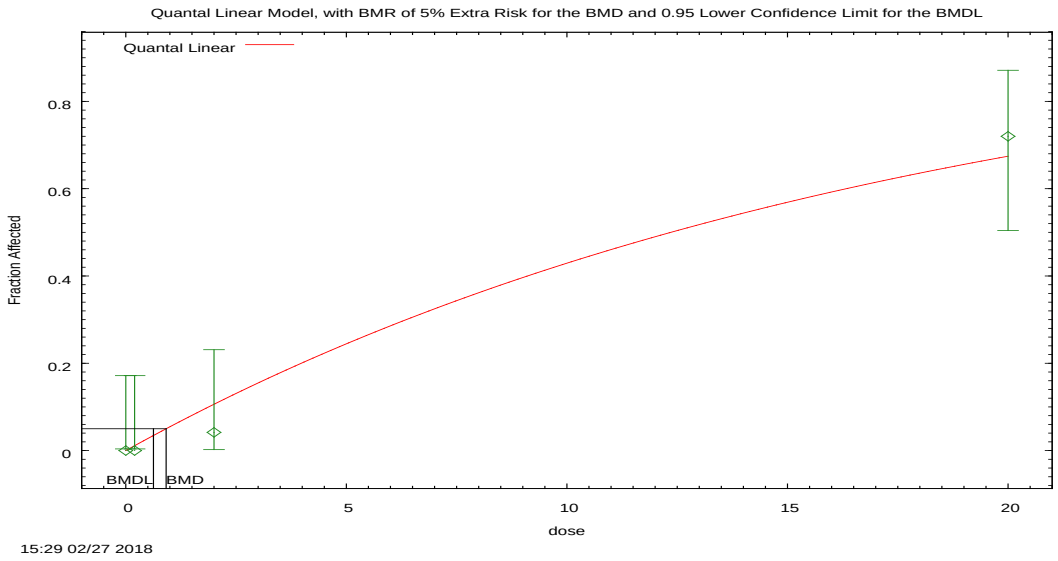


Figure D-2. Plot of incidence rate by dose, with fitted curve for Quantal-Linear model, for convulsions in female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage on gestational days (GDs) 6-19 (Cholakis et al., 1980); BMR = 5% ER; dose shown in mg/kg-day.

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 = 5% Extra risk

BMD = 0.914694

BMDL at the 95% confidence level = 0.627577

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0.0384615
Slope	0.056077	0.0588587
Power	n/a	1

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−18.98	4			
Fitted model	−20.04	1	2.11537	3	0.55
Reduced model	−47.98	1	57.9972	3	<.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	−1.02
20	0.6742	16.856	18	25	0.49

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

Table D-5. Model predictions for combined incidence of convulsion and mortality in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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 nonmultistage 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°[b]	**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-d 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-d, respectively; the BMD_{05} and $BMDL_{05}$ values for the selected model were 4.41 and 2.25 mg/kg-d, respectively.

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

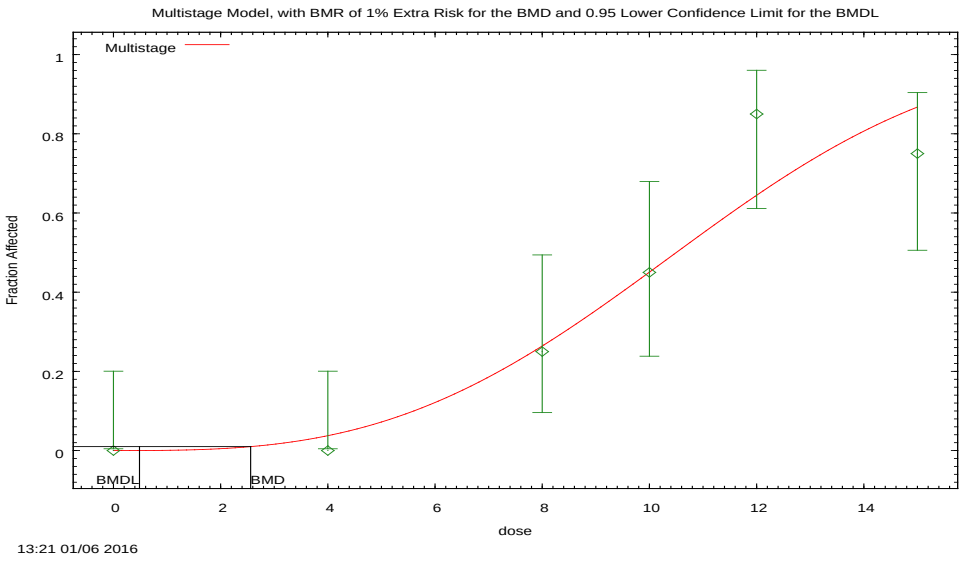


Figure D-3. Plot of incidence rate by dose, with fitted curve for Multistage 3° model, for combined incidence of convulsion and mortality in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER; dose shown in mg/kg-day.

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

Urinary System (Bladder) Effects

Table D-6. Model predictions for hemorrhagic/suppurative cystitis of the urinary bladder in male F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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	0.373	87.850	21.3	11.3	The Quantal-Linear model did not achieve an adequate fit (goodness-of-fit *p*-value <0.10). Of the remaining multistage models, the Multistage 3° model was selected based on lowest AIC. From among the Multistage 3° and nonmultistage models, the Multistage 3° model was selected based on lowest AIC.
Logistic	0.394	86.310	19.2	15.6	
LogLogistic	0.373	87.850	22.9	11.1	
Probit	0.321	86.683	16.8	13.6	
LogProbit	0.373	87.850	19.5	10.7	
Weibull	0.373	87.850	24.3	11.5	
Multistage 3°	**0.543**	**85.909**	**20.0**	**11.6**	
Multistage 2°	0.343	87.038	14.5	10.1	
Quantal-Linear	0.0181	95.014	7.00	4.86	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-d were −0.98, 1.06, 0.09, −0.21, 0.02, respectively.

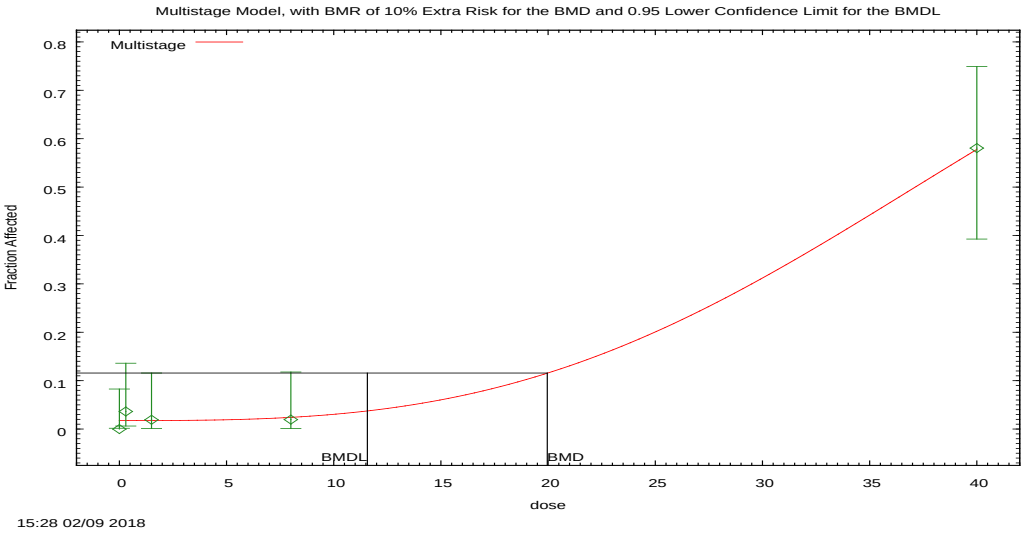


Figure D-4. Plot of incidence rate by dose, with fitted curve for Multistage 3° model, for hemorrhagic/suppurative cystitis of the urinary bladder in male F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Levine et al., 1983); BMR = 10% ER; dose shown in mg/kg-day.

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 = 10% Extra risk

BMD = 19.9607

BMDL at the 95% confidence level = 11.5693

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0175684	0.0172098
Beta(1)	0	0
Beta(2)	0	0
Beta(3)	0.0000132481	0.0000133069

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−39.54	5			
Fitted model	−40.95	2	2.83327	3	0.42
Reduced model	−73.82	1	68.5588	4	<.0001

AIC = 85.9086

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0176	0.949	0	54	−0.98
0.3	0.0176	0.966	2	55	1.06
1.5	0.0176	0.916	1	52	0.09
8	0.0242	1.235	1	51	−0.21
40	0.5792	17.955	18	31	0.02

χ^2 = 2.15 d.f. = 3 *p*-value = 0.5428

Prostate Effects

Table D-7 (and Figure D-5) 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-7. Model predictions for prostate suppurative inflammation in male F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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[c]	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-d 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-d, respectively; the BMD_{01} and $BMDL_{01}$ values for the selected model were 0.137 and 0.00906 mg/kg-d, 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.

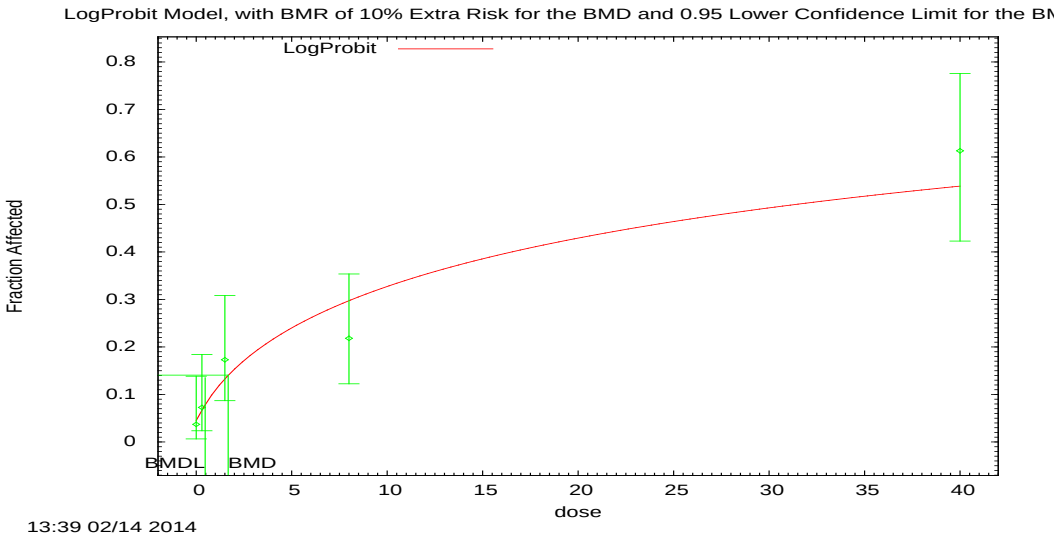


Figure D-5. Plot of incidence rate by dose, with fitted curve for the Log-Probit model, for prostate suppurative inflammation in male F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Levine et al., 1983); BMR = 10% ER; dose shown in mg/kg-day.

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% Extra risk

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

Mortality: Dose-Response Analysis and Benchmark Dose (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-8).

Table D-8. Mortality data selected for dose-response modeling for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
Lish et al. (1984b) (mortality at 11 wk)	$B6C3F_1$ mouse/male	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. (1984a) (mortality at 11 wk)	$B6C3F_1$ mouse/female	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]	F344 rat/female	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%)
	F344 rat/male	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%)
	F344 rat/male and female, 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)	CD rat/female	0 mg/kg-d 5 16 50	0/22 (0%) 0/22 (0%) 0/22 (0%) 6/22 (27%)

Table D-8. Mortality data selected for dose-response modeling for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
Levine et al. (1983) (mortality at 13 wk)	F344 rat/male	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%)
	F344 rat/female	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)	F344 rat/male	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%)
	F344 rat/female	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%)
Crouse et al. (2006)	F344 rat/female	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%)
	F344 rat/male	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%)
	F344 rat/male and female, 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%)

Table D-8. Mortality data selected for dose-response modeling for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (continued)

Reference	Species/sex	Dose	Incidence/total (%) or mean ± SD (number of animals)
Cholakis et al. (1980) (gestational exposure)	F344 rats/female (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)	SD rat/female (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)	New Zealand White rabbit/female (gestational exposure)	0 mg/kg-d 0.2 2 20	0/11 (0%) 0/11 (0%) 0/11 (0%) 0/12 (0%)

SD = standard deviation.

[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 death was reported in the 15 mg/kg-d dose group. However, this death was most likely not related to RDX, so the animal that died was excluded.

[c]For Cholakis et al. (1980) (13-week study in female rats), one accidental death was reported in the 0 mg/kg-d dose group. The animal that died was excluded.

Tables D-9 to D-12 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 data sets were not modeled because each had either no response or a positive response only in the highest dose group: 11-week mortality data from Lish et al. (1984b), 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-9. Model predictions for combined mortality in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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 nonmultistage 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-d 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-d, respectively; the BMD_{05} and $BMDL_{05}$ estimates for the selected model were 32.4 and 11.0 mg/kg-d, respectively.

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

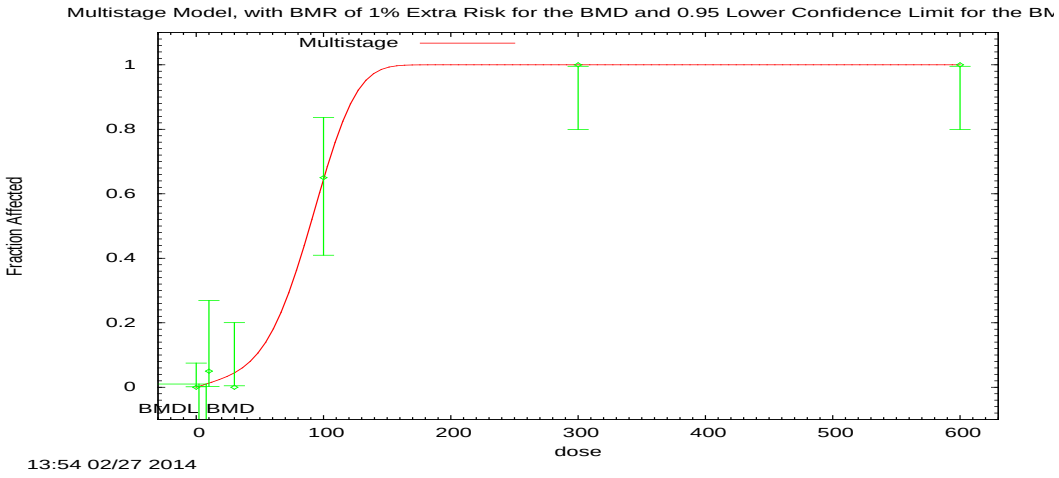


Figure D-6. Plot of incidence rate by dose, with the fitted curve of the Multistage 2° model, for combined mortality in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 13 weeks (Levine et al., 1981b); BMR = 1% ER; dose shown in mg/kg-day.

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 = 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

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

Table D-10. Model predictions for mortality (number found dead) in rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the diet for 13 weeks (von Oettingen et al., 1949); 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	0.0341	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 achieved 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°[a]	0.0351	66.517	1.22	0.628	
Quantal-Linear	0.0995	64.639	0.919	0.623	

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

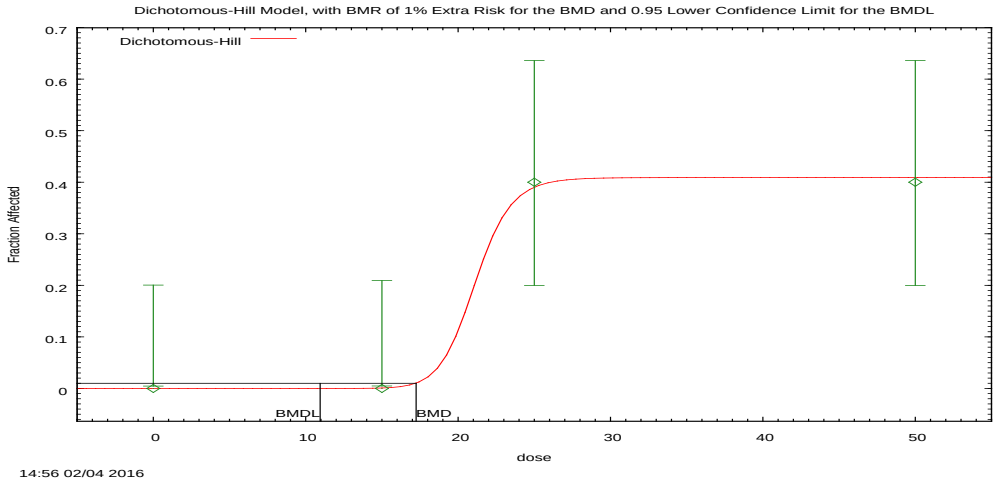


Figure D-7. Plot of incidence rate by dose, with fitted curve for Dichotomous-Hill model, for mortality (number found dead) in rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the diet for 13 weeks (von Oettingen et al., 1949); BMR = 1% ER; dose shown in mg/kg-day.

Table D-11. Model predictions for combined mortality (number found dead) in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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 nonmultistage 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°[b]	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-d 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 4° and Multistage 5° models may appear to be equivalent; however, differences exist in digits not displayed in the table.

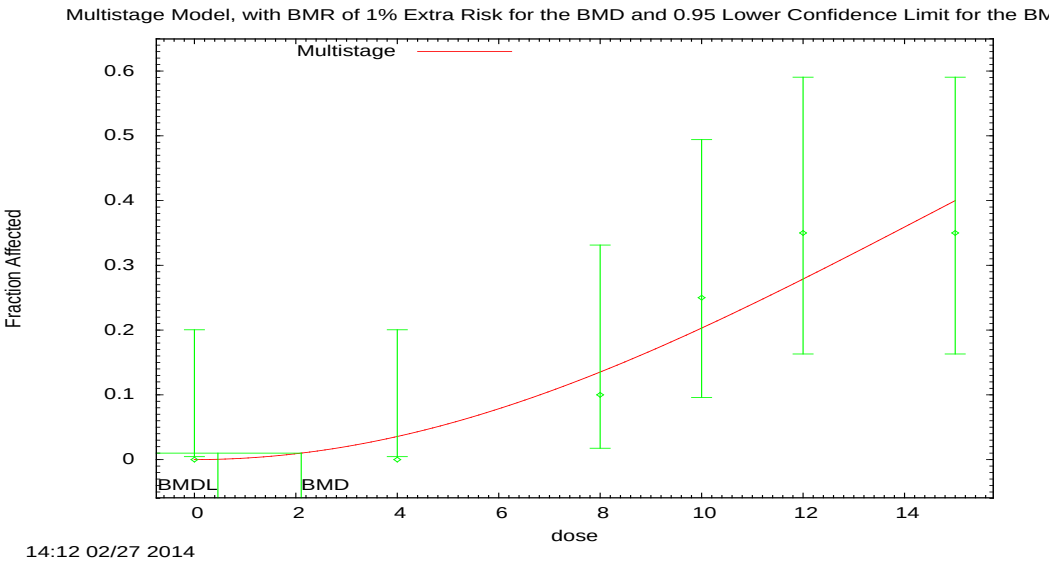


Figure D-8. Plot of incidence rate by dose, with the fitted curve of the Multistage 2° model, for mortality in male and female F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER; dose shown in mg/kg-day.

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

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

Table D-12. Model predictions for mortality in female Sprague-Dawley rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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 nonmultistage 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-d 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-d, respectively; the BMD_{05} and $BMDL_{05}$ estimates for the selected model were 5.23 and 7.29 mg/kg-d, respectively.

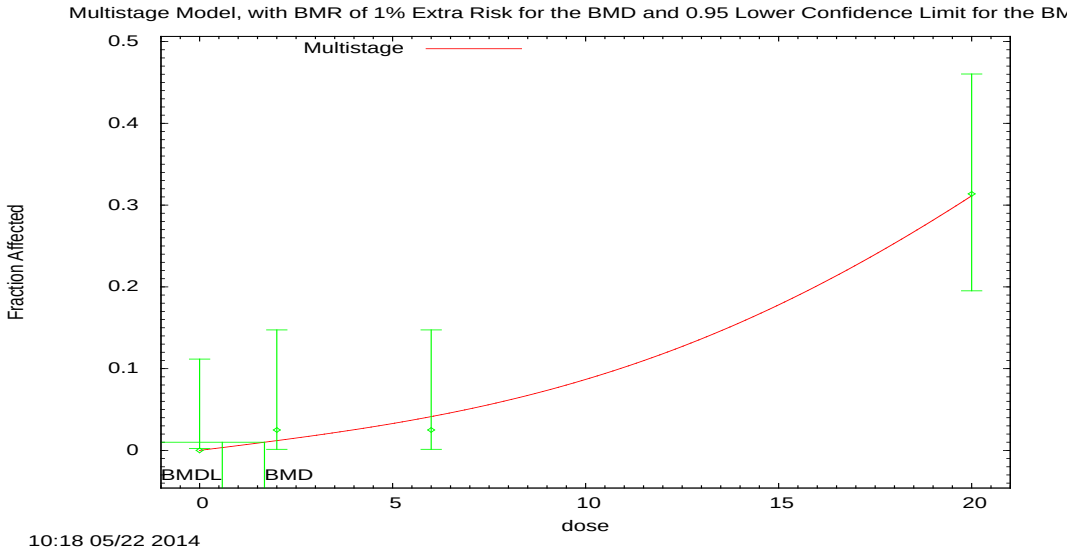


Figure D-9. Plot of incidence rate by dose, with the fitted curve of the Multistage 3° model, for mortality in female Sprague-Dawley rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by gavage on Gestation Days 6–15 (Angerhofer et al., 1986); BMR = 1% ER; dose shown in mg/kg-day.

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 = 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-13. For each endpoint, the doses and tumor incidence data used for the modeling are presented.

Table D-13. Cancer endpoints selected for dose-response modeling for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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

PWG = Pathology Working Group.
[a]The highest dose group (175/100 mg/kg-d) was excluded from the analysis because approximately half the animals in the group died from overdosing, which possibly introduces bias in the estimate of response rate in that group. See Section 2.3.1 for more details.
[b]For female mouse hepatocellular tumors from Lish et al. (1984b), tumor incidence and totals are those reported in the PWG re-evaluation (Parker et al., 2006).

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

First, the survival curves were compared across dose groups for female mice in Lish et al. (1984b) 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 the high-dose group. The test yielded a nonsignificant result (p-value = 0.64), 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 χ^2 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

[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 (2012) is used for selecting cancer models because the model family (multistage) is selected a priori.

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 the MS-COMBO procedure in BMDS, which calculates the BMD and BMDL for combinations of tumors under the assumption that the tumor incidences are mutually independent. Bogen and Seilkop (1993) analyzed the correlation among tumor rates in treated and untreated animals from 24 previously published studies and observed that all correlations among the rates of different tumor types were low. Based on these findings, NRC (1994) noted that the assumption of independence in incidence between tumor types is reasonable when no evidence exists to the contrary. Because there is no evidence that tumor types related to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure are correlated, it is reasonable to assume that liver and lung tumor rates subsequent to RDX exposure are independent.

In its analysis of combined tumor data, the MS-COMBO procedure does not rely on the modeling of pooled incidence of "tumor-bearing" animals (i.e., animals with one or more tumors). As noted by NRC (1994) and Bogen (1990), an approach based on such a pooled incidence does not reflect possible differences in dose-response relationships across sites and would tend to underestimate the overall risk of tumor incidence when tumors occur independently across sites. Instead, the procedure does a "composite potency analysis," as described in Bogen (1990). Specifically, the combined incidence model is based on the probability of combined incidence across tumor types as derived using the multistage model for the individual incidence of each tumor type and the assumption of independence. This model has the multistage form:

$$P(x) = 1 - \exp[-(\beta_0 + \beta_1 x + \beta_2 x^2 + \cdots)] \quad \text{(D-1)}$$

where the coefficients β_j are specified by

$$\beta_0 = \sum_{i=1}^{t} \beta_{0i}, \beta_1 = \sum_{i=1}^{t} \beta_{1i}, \beta_2 = \sum_{i=1}^{t} \beta_{2i}, \text{ etc.,}$$

t is the number of tumors under consideration, and β_{xi} is the xth parameter (x = 0, 1, 2,...) for tumor i. In the case of Lish et al. (1984b), there are two tumor types, so $t = 2$. Note that the degree of multistage model used in the combined analysis can vary across sites.

D.2.2. Modeling Results for Female Mouse Tumor Data

The BMD modeling results for mouse tumor data sets are provided in Tables D-14 to D-16 (and Figures D-10 to D-12).

Mouse Tumor Data— Benchmark Dose (BMD) Modeling Results

Table D-14. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with highest dose dropped; 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°**	**0.375**	**184.58**	**29.9**	**14.9**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were 0.68, −1.12, 0.48, −0.06, respectively.
[b]For the Multistage 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

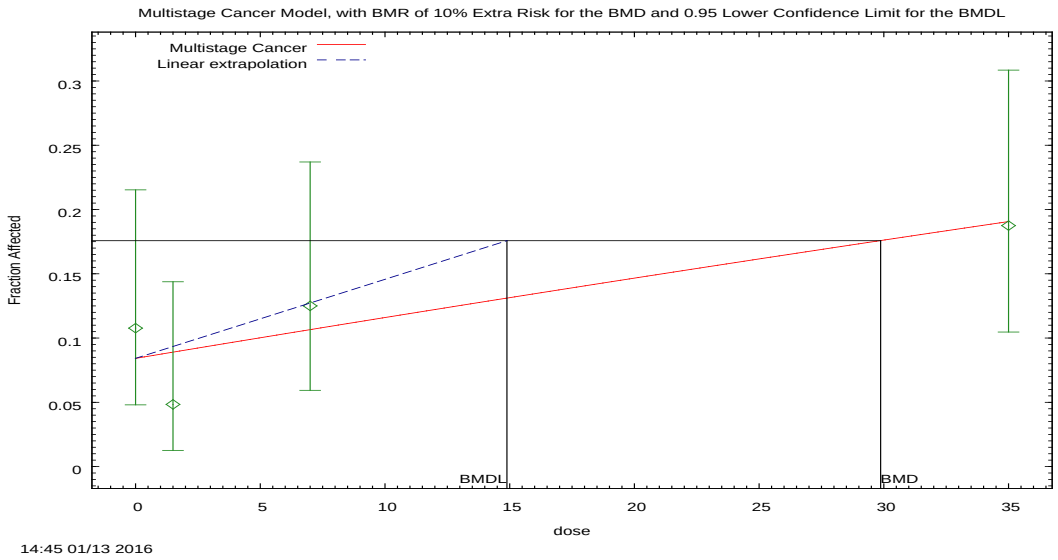


Figure D-10. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 1° model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 29.8728

BMDL at the 95% confidence level = 14.8898

Benchmark dose upper bound (BMDU) at the 95% confidence level = 169.654

Taken together, (14.8898, 169.654) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00671603

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0841575	0.084635
Beta(1)	0.00352698	0.00347086

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−89.22	4			
Fitted model	−90.29	2	2.14531	2	0.34
Reduced model	−92.36	1	6.29109	3	0.1

AIC = 184.582

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0842	5.47	7	65	0.68
1.5	0.089	5.517	3	62	−1.12
7	0.1065	6.815	8	64	0.48
35	0.1905	12.193	12	64	-0.06

$\chi^2 = 1.96$ d.f. = 2 p-value = 0.3749

Table D-15. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped; 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]	**0.390**	**136.52**	**25.5**	**14.2**	All of the models reduced to the Multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were −0.97, 0.82, 0.47, −0.23, respectively.

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

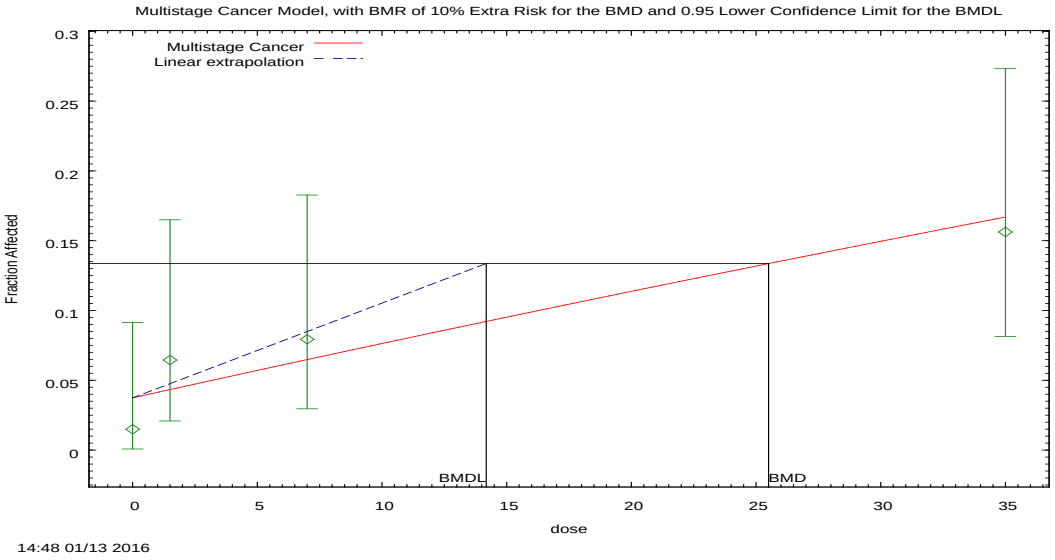


Figure D-11. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 1° model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 25.5021

BMDL at the 95% confidence level = 14.1795

BMDU at the 95% confidence level = 68.9086

Taken together, (14.1795, 68.9086) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00705244

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0374026	0.0421855
Beta(1)	0.00413144	0.00372219

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−65.23	4			
Fitted model	−66.26	2	2.05455	2	0.36
Reduced model	−70.19	1	9.91138	3	0.02

AIC = 136.516

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0374	2.506	1	67	−0.97
1.5	0.0433	2.688	4	62	0.82
7	0.0648	4.085	5	63	0.47
35	0.167	10.688	10	64	−0.23

$\chi^2 = 1.88$ d.f. = 2 P-value = 0.3902

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, with highest dose dropped; BMR = 10% ER

BMD = 13.8 mg/kg-day; BMDL = 8.53 mg/kg-day

MS-COMBO Results

BMR of 10% Extra Risk

```
**** Start of combined BMD and BMDL Calculations.****
Combined Log-Likelihood                    -156.54886486624446
  Combined Log-likelihood Constant             140.87841237048966

   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        13.7575
            BMDL =        8.53099
Multistage Cancer Slope Factor =      0.011722
```

Analysis to Address Discrepancy in Sample Size

As reported in Parker et al. (2006), the Pathology Working Group (PWG)'s re-evaluation of female mouse liver tumors from Lish et al. (1984b) yielded different sample sizes in two dose groups than did the original study, a discrepancy that could not be resolved (see Table 1-13 of the Toxicological Review). To determine how this discrepancy affected the modeling results, the liver tumor data were remodeled using the incidence frequencies from Parker et al. (2006) and the sample sizes from Lish et al. (1984b). The BMD model results from this reanalysis are provided in Table D-16, along with the plot and output for the selected model. The BMD and BMDL from this reanalysis were 25.7 and 14.3 mg/kg-day, respectively, which are close to the BMD and BMDL (25.5 and 14.2 mg/kg-day, respectively) from the original analysis using incidence data from Parker et al. (2006). In addition, MS-COMBO was also run with the results from the liver tumor reanalysis combined with the BMD model results for lung tumors. The BMD and BMDL from this combined analysis were 13.8 and 8.56 mg/kg-day, which were close to the BMD and BMDL (13.8 and 8.53 mg/kg-day, respectively) from the original MS-COMBO analysis. Therefore, it was determined that the sample size discrepancy had little effect on the BMDL and consequently the oral slope factor (OSF).

Table D-16. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped and sample sizes from Lish et al. (1984b); 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°**	**0.413**	**136.50**	**25.7**	**14.3**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were −0.95, 0.8, 0.43, −0.22, respectively.

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

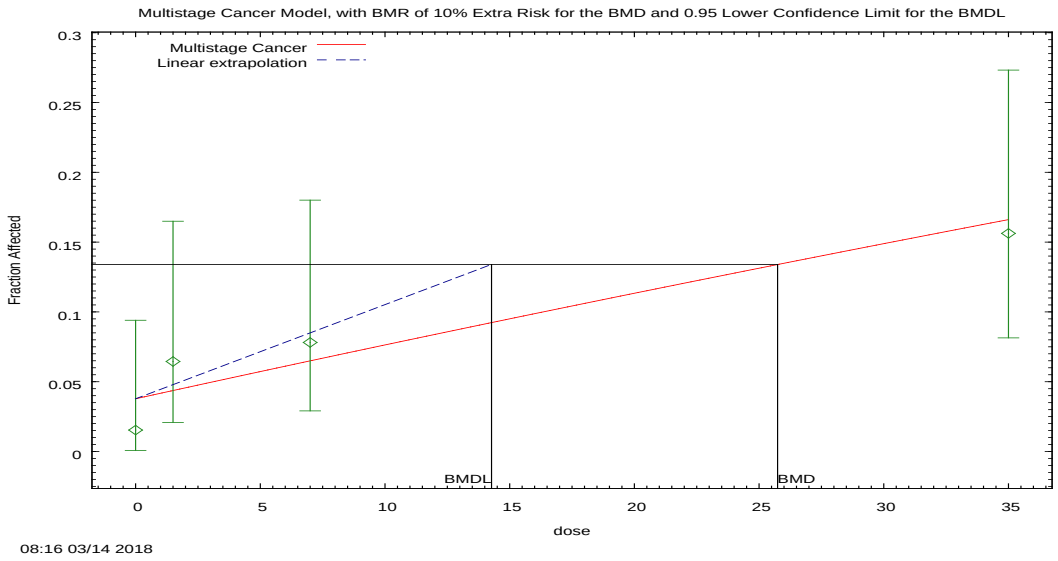


Figure D-12. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 1° model, for combined hepatocellular adenoma and carcinoma in female B6C3F1 mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped and sample sizes from Lish et al. (1984b); BMR = 10% ER; dose shown in mg/kg-day.

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 = 25.7422

BMDL at the 95% confidence level = 14.2717

BMDU at the 95% confidence level = 70.4126

Taken together, (14.2717, 70.4126) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00700686

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0378291	0.041973
Beta(1)	0.00409291	0.00372237

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−65.28	4			
Fitted model	−66.25	2	1.93249	2	0.38
Reduced model	−70.1	1	9.6454	3	0.02

AIC = 136.497

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0378	2.459	1	65	−0.95
1.5	0.0437	2.711	4	62	0.8
7	0.065	4.16	5	64	0.43
35	0.1662	10.64	10	64	−0.22

$\chi^2 = 1.77$ d.f. = 2 P-value = 0.413

MS-COMBO Results

BMR of 10% Extra Risk

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

  Combined Log-Likelihood                      -156.53930900047271

  Combined Log-likelihood Constant              140.92945266044825


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         13.827

            BMDL =        8.56197

Multistage Cancer Slope Factor =     0.0116796
```

D.2.3. Additional Dose-Response Analysis: Male Mice and Rats

This appendix also presents a quantitative dose-response analysis of incidence of lung carcinomas in male B3C6F$_1$ mice and incidence of liver carcinomas in male F344 rats (Levine et al., 1983) (see Table D-17). The resulting candidate OSFs are presented for comparison with OSF estimates provided in Section 2.3.3 of the Toxicological Review.

Table D-17. Carcinoma data from Lish et al. (1984b) and Levine et al. (1983)

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

[a]The highest dose (175/100 mg/kg-d) was excluded from the analysis because approximately half the animals in the group died from overdosing, which possibly introduces bias in the estimate of response rate in that group. See Section 2.3.1 for more details.

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

Male Mouse Lung Tumor Analysis

For male mice in Lish et al. (1984b), a log-rank test on the Kaplan-Meier survival curves with the highest dose dropped, stratified by dose, yielded a nonsignificant result (p-value = 0.50), 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. (1984b). A non-time-dependent dose-response analysis was conducted using BMDS multistage-cancer models with the highest dose dropped, 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 scaling to the ¾ power ($BW^{3/4}$) (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-18; detailed BMD modeling results are provided in Table D-19 (and Figure D-13).

Table D-18. Model predictions and oral slope factor for alveolar/bronchiolar carcinomas in male B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 2 years (Lish et al., 1984b)

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	42.5	24.7	3.69	0.027

BW_a = animal body weight; BW_h = human body weight

[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-19. Model predictions for alveolar/bronchiolar carcinoma in male B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 2 years (Lish et al., 1984b), with highest dose dropped; 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 3°	**0.402**	**135.85**	**42.5**	**24.7**	The Multistage 3° model was selected based on lowest AIC.
Multistage 2°	0.391	135.90	47.1	24.5	
Multistage 1°	0.360	136.10	65.9	23.9	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were −0.55, 1.11, −0.54, 0.01, respectively.

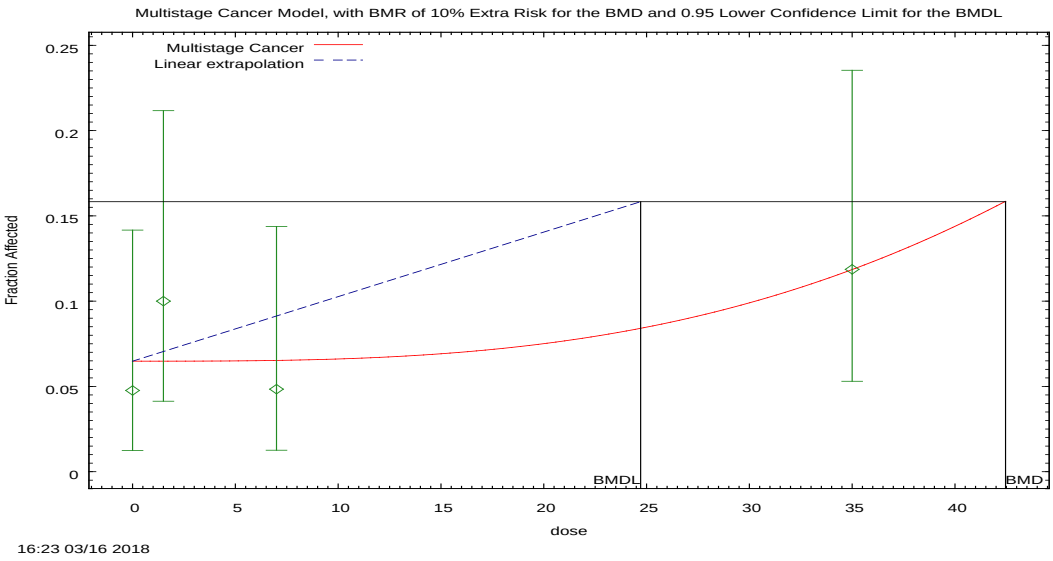


Figure D-13. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 3° model, for alveolar/bronchiolar carcinoma in male B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 42.459

BMDL at the 95% confidence level = 24.7112

BMDU at the 95% confidence level = 81368.9

Taken together, (24.7112, 81368.9) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00404674

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0647969	0.0655597
Beta(1)	0	0
Beta(2)	0	0
Beta(3)	1.3765E-06	1.3607E-06

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−65.07	4			
Fitted model	−65.92	2	1.71424	2	0.42
Reduced model	−66.74	1	3.35142	3	0.34

AIC = 135.847

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0648	4.082	3	63	−0.55
1.5	0.0648	3.888	6	60	1.11
7	0.0652	4.045	3	62	−0.54
35	0.1184	6.985	7	59	0.01

$\chi^2 = 1.82$ d.f. = 2 *p*-value = 0.4021

Male Rat Liver Tumor Analysis

For male rats in the Levine et al. (1983) study, 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 it. 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 precluded the matching of the times of death and the tumor incidence of the animals, thus precluded 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-17). 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 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, 2005).

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-20; detailed BMD modeling results are provided in Table D-21 (and Figure D-14). 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-20. Model predictions and oral slope factor for hepatocellular carcinomas in male F344 rats administered hexahydro-1,3,5-trinitro-1,3,5-triazine (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]

BW_a = animal body weight; BW_h = human body weight

[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-21. Model predictions for combined hepatocellular adenoma and carcinoma in F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (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-d 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.

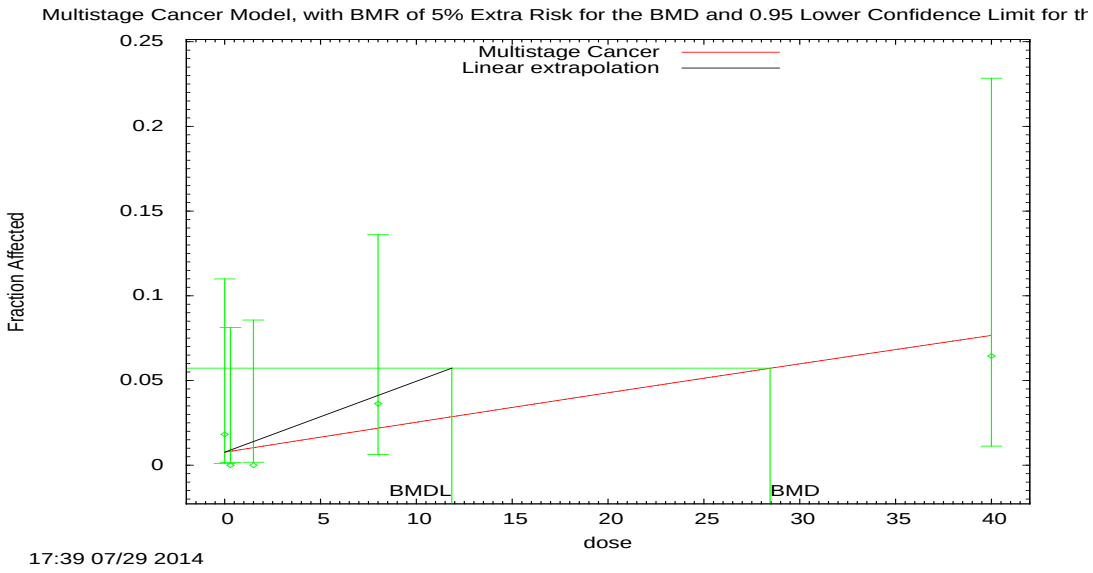


Figure D-14. Plot of incidence rate by dose, with fitted curve for Multistage 1° model, for combined hepatocellular adenoma and carcinoma in F344 rats exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Levine et al., 1983); BMR = 5% ER; dose shown in mg/kg-day.

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% extra risk

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

D.2.4. Sensitivity Analysis on Dose-Response Modeling of Female Mouse Tumor Data

Comparison of Multistage and Nonmultistage Model Fits with and without High Dose

In their evaluation of the external review draft of the RDX assessment (SAB, 2017), the Science Advisory Board (SAB) expressed concern about the fit of the multistage cancer models to the mouse liver and lung tumor data from Lish et al. (1984b) and Parker et al. (2006). In the external review draft assessment, all the dose groups were included in the dose-response modeling. On p. 72, the SAB report stated,

> The SAB expresses concern that the near linearity of the fitted multistage dose-response models…results in a relatively poor fit (model estimates) at the highest doses. The two fitted models used…have $BMDL_{10}$ estimates that are larger

> than the two lowest nonzero doses used. The Cancer Guidelines (USEPA, 2005) states (page 3-16): '*If the POD is above some data points, it can fail to reflect the shape of the dose-response curve at the lowest doses and can introduce bias into subsequent extrapolations.*' This seems to be what is happening with the data on RDX-induced adenomas and carcinomas, and the issues with the $BMDL_{10}$ seem to arise primarily because the fitted multistage models (with parameter constraints invoked) lack sufficient curvature. Larger than expected $BMDL_{10}$ values (the PODs) result in lower estimated OSFs. The SAB conjectures that using a model form that allows more curvature could provide a better fit at the mid-range and higher doses, and improve the quality of fit. As mentioned in Section 3.3.5.3, the SAB acknowledges that EPA's standard practice is to use the multistage model for benchmark dose modeling of cancer dose-response when there is no biological basis for choosing another model. In this case however, the relatively poor fit of the multistage model to the hepatocellular and alveolar/bronchiolar adenomas and carcinomas data produces an estimate of the POD with poor properties. The SAB recommends that at a minimum, the assessment discuss the adequacy of the fit of the multistage model to available data. This discussion could be further supported by exploring and reporting fits to other standard BMD model forms—engaging in a curve-fitting exercise starting for example with the list in Table D-13 in the draft supplemental document. Although the multistage model does ensure positive slopes throughout, the BMDS software facilitates fitting other models that also adhere to this constraint.

In its key recommendations (p. 65), the SAB also stated that "the effect of including/excluding the highest dose on model choice and the POD estimate" should be explored. In response to these recommendations, the liver and lung tumor data were modeled using multistage and nonmultistage models, both with all doses included and with highest dose group dropped.

Analysis of Female Mouse Liver Tumor Data

Table D-22 summarizes the BMD model results for liver tumors for the case where all doses are included, along with the plot and output for the Multistage 1° model,[8] which was selected for calculating the OSF in the external review draft of the assessment. The Multistage 1° model exhibited an adequate fit to the data. Its goodness-of-fit *p*-value was above 0.10, and its residuals were all below 2 in absolute value. However, as demonstrated by the plot (see Figure D-15), it did not account for the supralinearity (steep rise) in the data near the origin, and the residual at the control group (−1.37) was moderately high. The Log-Probit model had a substantially lower AIC value than the Multistage 1° model and the other models; the plot for this model is provided in Figure D-16, followed by its output. As demonstrated by the plot, the Log-Probit model accounted for supralinearity near the origin, and the residual for the control group was very low. Therefore, this model exhibited a better fit to the data than the multistage models. However, the BMDL for this

[8]Throughout this sensitivity analysis, the term "Multistage 1°" is used to describe the Quantal-Linear model in order to conform to the terminology used in cancer modeling; the Multistage 1° and Quantal-Linear models are mathematically equivalent. (In many cases, the term "quantal linear" appears in BMDS output.)

model was exceedingly low, and the BMD:BMDL ratio was approximately 67, indicating substantial uncertainty in the BMD estimate. Table D-23 presents the BMD model results for multistage and nonmultistage models for liver tumors, with the highest dose dropped. Also presented are the plot (see Figure D-17) and output for the Multistage 1° model that was selected for calculating the OSF in the external review draft of this assessment. With the highest dose dropped, the Multistage 1° model had an AIC value that was close to the lowest among the models, and the residuals for all the dose groups, including control, were below 1 in absolute value. Therefore, the quality of fit of the Multistage 1° model was comparable to the best-fitting model among the nonmultistage models.

Table D-22. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with all doses included; BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)
	p-value	AIC		
Gamma	0.160	164.06	64.2	32.6
Logistic	0.0849	165.59	95.7	59.2
LogLogistic	0.178	163.81	59.1	28.5
Probit	0.0917	165.41	91.7	55.2
LogProbit	0.678	161.08	19.1	0.286
Weibull[b] **Multistage 1°[c]**	**0.160**	**164.06**	**64.2**	**32.6**
Multistage 4°[d] Multistage 3° Multistage 2°[e]	0.160	164.06	64.2	32.6

[a]Multistage model selected in external review draft assessment in bold.
[b]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Multistage 1° model.
[c]The Multistage 1° model may appear equivalent to the Gamma model, however differences exist in digits not displayed in the table.
[d]For the Multistage 3° and 4° models, the b3 and b4 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model.
[e]The Multistage 2° model may appear equivalent to the Gamma and Weibull models, however differences exist in digits not displayed in the table.

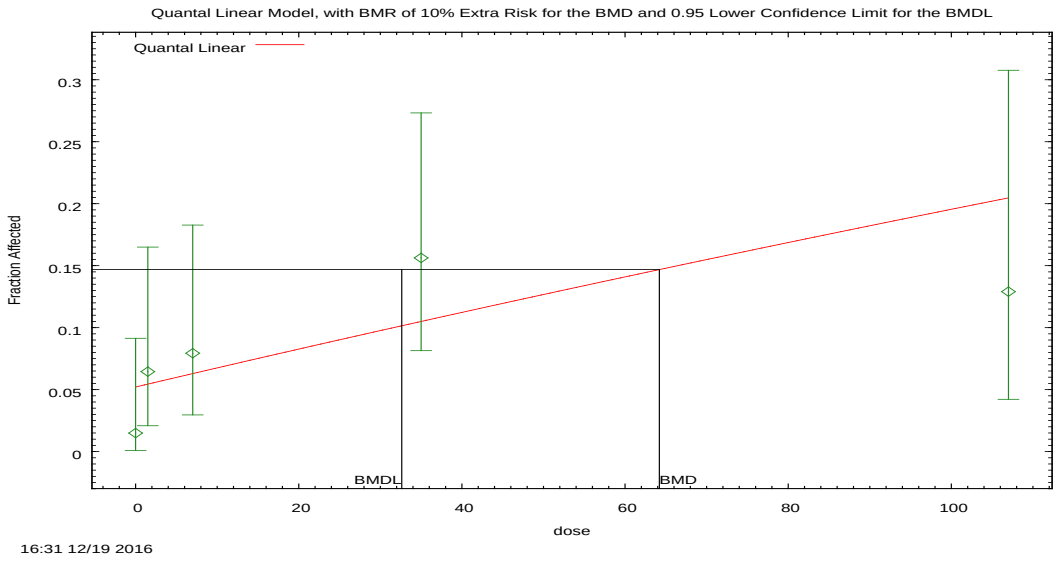


Figure D-15. Plot of incidence rate by dose, with fitted curve for Multistage 1° model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with all doses included; BMR = 10% ER; dose shown in mg/kg-day.

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 = 10% Extra risk

BMD = 64.2024

BMDL at the 95% confidence level = 32.6282

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0520756	0.0289855
Slope	0.00164107	0.00126065
Power	n/a	1

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−77.15	5			
Fitted model	−80.03	2	5.75967	3	0.12
Reduced model	−82.52	1	10.74	4	0.03

AIC = 164.063

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0521	3.489	1	67	−1.37
1.5	0.0544	3.373	4	62	0.35
7	0.0629	3.963	5	63	0.54
35	0.105	6.719	10	64	1.34
107	0.2047	6.347	4	31	−1.04

$\chi^2 = 5.17$ d.f. = 3 p-value = 0.16

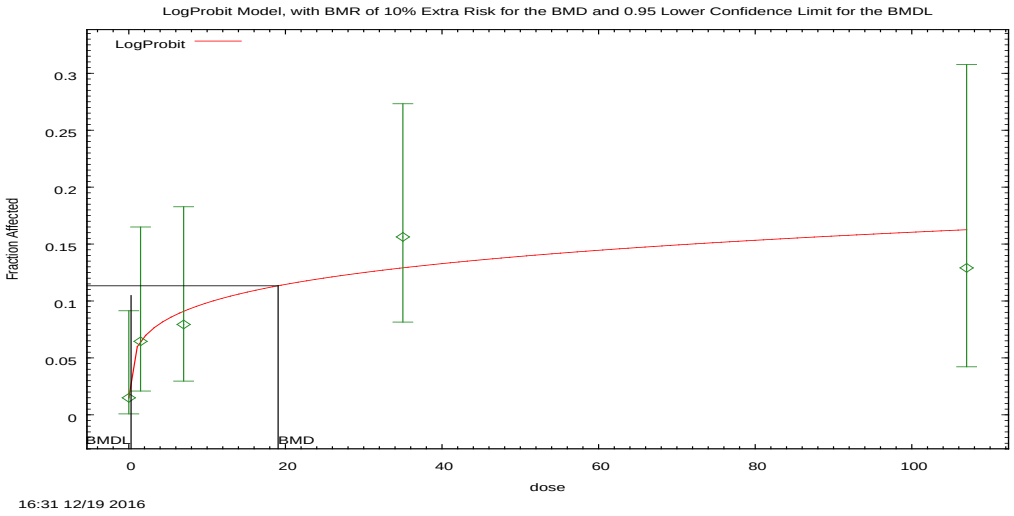


Figure D-16. Plot of incidence rate by dose, with fitted curve for Log-Probit model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with all doses included; BMR = 10% ER; dose shown in mg/kg-day.

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% extra risk

BMD = 19.0651

BMDL at the 95% confidence level = 0.285889

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0147757	0.0149254
Intercept	−1.6996E+00	−1.7126E+00
Slope	0.141823	0.143382

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−77.15	5			
Fitted model	−77.54	3	0.775228	2	0.68
Reduced model	−82.52	1	10.74	4	0.03

AIC = 161.078

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0148	0.99	1	67	0.01
1.5	0.0643	3.988	4	62	0.01
7	0.0909	5.727	5	63	−0.32
35	0.129	8.258	10	64	0.65
107	0.1624	5.036	4	31	−0.5

$\chi^2 = 0.78$ d.f. = 2 p-value = 0.6777

Table D-23. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped and multistage and nonmultistage models fitted; BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)
	p-value	AIC		
Gamma Multistage 3°[b] Multistage 2°	0.390	136.52	25.5	14.2
Logistic	0.319	137.15	31.0	22.6
LogLogistic	0.400	136.44	24.6	13.0
Probit	0.327	137.08	30.4	21.4
LogProbit	0.645	136.68	13.7	1.66
Weibull[c] **Multistage 1°[d]**	**0.390**	**136.52**	**25.5**	**14.2**

[a]Multistage models used in cancer assessment in bold.
[b]For the Multistage 3° model, the beta coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model.
[c]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Multistage 1° model.
[d]The Weibull and Multistage 1° models may appear equivalent to the Gamma, Multistage 2°, Multistage 3° models, however differences exist in digits not displayed in the table.

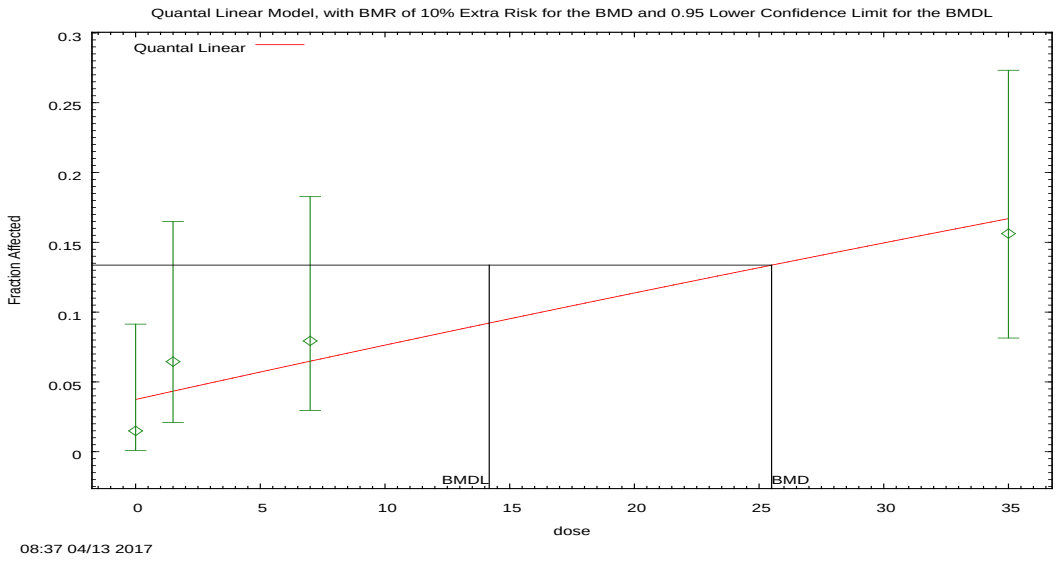


Figure D-17. Plot of incidence rate by dose, with fitted curve for Multistage 1° model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 10% extra risk

BMD = 25.5022

BMDL at the 95% confidence level = 14.1795

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0374028	0.0289855
Slope	0.00413143	0.00436879
Power	n/a	1

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−65.23	4			
Fitted model	−66.26	2	2.05455	2	0.36
Reduced model	−70.19	1	9.91138	3	0.02

AIC = 136.516

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0374	2.506	1	67	−0.97
1.5	0.0433	2.688	4	62	0.82
7	0.0648	4.085	5	63	0.47
35	0.167	10.688	10	64	-0.23

χ^2 = 1.88 d.f. = 2 *p*-value = 0.3902

Analysis of Female Mouse Lung Tumor Data

Tables D-24 and D-25 (and Figures D-18 and D-19) present the BMD model results for multistage and nonmultistage models for lung tumors, with all doses included and with the highest dose dropped, respectively. Also presented for each case is the plot and output for the Multistage 1° model. For the case with all doses included, the AIC value for the Multistage 1° model was near the lowest from among the models, so its fit was comparable to the best-fitting model. For the case with the highest dose dropped, the Multistage 1° model had the lowest AIC value of all the models, so it was the best-fitting model. Therefore, in both cases the multistage model yielded a quality of fit that was comparable to the best-fitting model.

Table D-24. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with all doses included and multistage and nonmultistage models fitted; BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)
	p-value	AIC		
Gamma[b] Multistage 4°[c] Multistage 3°[c] Multistage 2°[d]	0.417	218.68	52.8	27.7
Logistic	0.315	219.45	70.4	45.6
LogLogistic	0.439	218.53	49.2	24.2
Probit	0.328	219.34	67.8	42.9
LogProbit	0.339	219.93	37.0	10.2
Weibull[e] Multistage 1°	**0.417**	**218.68**	**52.8**	**27.7**

[a]Multistage model selected in external review draft assessment draft in bold.
[b]The Gamma model may appear equivalent to the Weibull model, however differences exist in digits not displayed in the table.
[c]For the Multistage 3° and 4° models, the b3 and b4 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model.
[d]The Multistage 2° model may appear equivalent to the Weibull model, however differences exist in digits not displayed in the table. This also applies to the Multistage 1° model.
[e]For the Weibull model, the power parameter estimate was 1. The models in this row reduced to the Multistage 1° model.

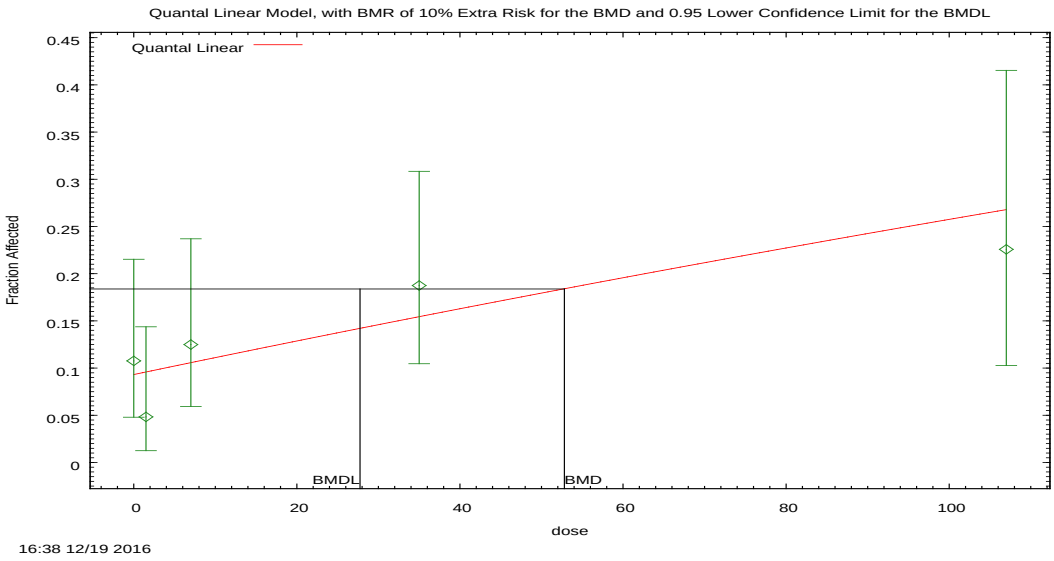


Figure D-18. Plot of incidence rate by dose, with fitted curve for Multistage 1° model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with all doses included; BMR = 10% ER; dose shown in mg/kg-day.

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 = 10% extra risk

BMD = 52.8079

BMDL at the 95% confidence level = 27.748

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0931678	0.119403
Slope	0.00199517	0.00140632
Power	n/a	1

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−105.78	5			
Fitted model	−107.34	2	3.12764	3	0.37
Reduced model	−110.16	1	8.77367	4	0.07

AIC = 218.682

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0932	6.056	7	65	0.4
1.5	0.0959	5.944	3	62	−1.27
7	0.1057	6.768	8	64	0.5
35	0.1543	9.877	12	64	0.73
107	0.2675	8.292	7	31	−0.52

$\chi^2 = 2.84$ d.f. = 3 p-value = 0.4168

Table D-25. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with highest dose dropped and multistage and nonmultistage models fitted; BMR = 10% ER

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)
	p-value	AIC		
Gamma[b]	0.161	186.58	30.0	14.9
Logistic	0.364	184.66	31.9	20.5
LogLogistic	0.162	186.57	29.7	13.9
Probit	0.366	184.65	31.7	19.7
LogProbit	0.171	186.47	28.4	9.50
Weibull[c]	0.161	186.58	30.0	14.9
Multistage 3°[d] **Multistage 2°** **Multistage 1°**	**0.375**	**184.58**	**29.9**	14.9

[a]Multistage model used in cancer assessment in bold.
[b]The Gamma model may appear equivalent to the Weibull model, however differences exist in digits not displayed in the table.
[c]The Weibull model may appear equivalent to the Gamma model, however differences exist in digits not displayed in the table.
[d]For the Multistage 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 1° model.

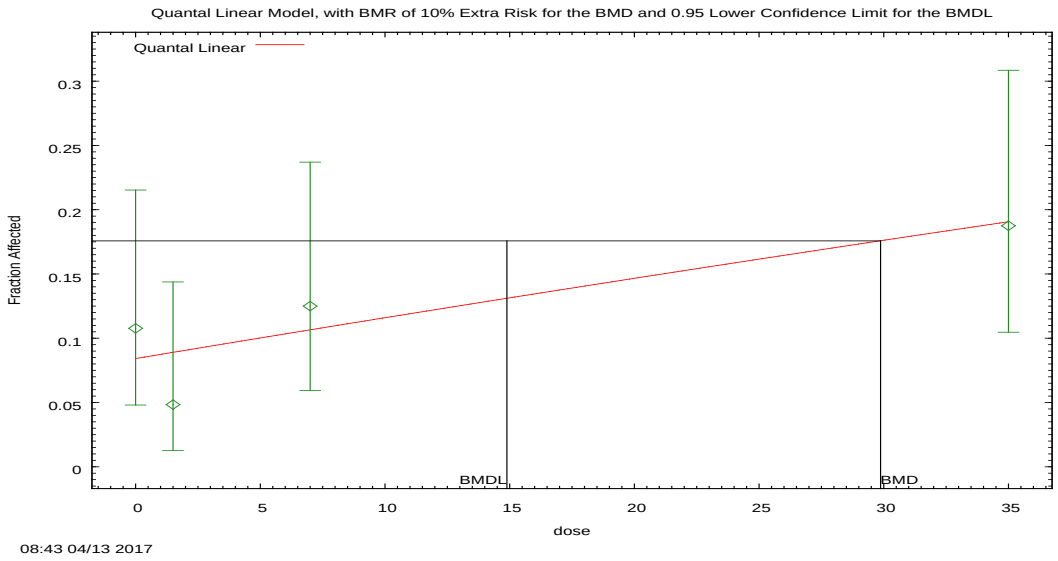


Figure D-19. Plot of incidence rate by dose, with fitted curve for Multistage 1° model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 10% extra risk

BMD = 29.8728

BMDL at the 95% confidence level = 14.8898

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0841575	0.119403
Slope	0.00352698	0.0026345
Power	n/a	1

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−89.22	4			
Fitted model	−90.29	2	2.14531	2	0.34
Reduced model	−92.36	1	6.29109	3	f0.1

AIC = 184.582

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0842	5.47	7	65	0.68
1.5	0.089	5.517	3	62	−1.12
7	0.1065	6.815	8	64	0.48
35	0.1905	12.193	12	64	−0.06

$\chi^2 = 1.96$ d.f. = 2 *p*-value = 0.3749

Discussion of Multistage and Nonmultistage Model Results for Female Mouse Tumor Data

Regarding the BMD model results on liver tumors with all doses included, the multistage models exhibit an inferior fit to the data compared to a subset of nonmultistage models (in this case, the Log-Probit), which would raise concerns about the appropriateness of their use. However, with the highest dose dropped, the multistage models yielded a fit comparable to the best-fitting model. For lung tumors, the multistage models exhibited fits comparable to the best-fitting model among multistage and nonmultistage models, both with all doses included and with the highest dose dropped.

As noted in the SAB report (SAB, 2017), EPA uses a linear low-dose extrapolation approach to estimate human cancer risk when the mode of carcinogenic action for tumor incidence is unknown, as is the case for RDX (see Section 2.3.2). Thus, the multistage models are used for modeling cancer data because they allow for the statistical plausibility of low-dose linearity, that is, a positive slope at the origin. When the estimate of the first-degree coefficient of the multistage model is positive, the slope of the estimated model is positive. Furthermore, even when the estimate of the first-degree coefficient is zero, the upper confidence bound on the slope is positive, thus allowing for the plausibility of low-dose linearity. This property does not hold for most of the other models in BMDS, where the slope at the origin is either zero or infinite for all possible parameter estimates (except in some cases when models reduce to the Multistage 1° model). With

the highest dose dropped, the multistage models were the best-fitting or near the best-fitting for both liver and lung tumors, and were determined to be sufficient for use for these data.

Analysis Using Female Mouse Historical Control Tumor Rates

In their evaluation of the external review draft of the RDX assessment (SAB, 2017), the SAB expressed concern that the liver tumor rate in the concurrent control was low compared to historical control. As indicated in Haseman et al. (1985), the liver tumor rate of untreated female B6C3F$_1$ mice in National Toxicology Program (NTP) studies had a mean of 8.3% across studies collected, while the control group in Lish et al. (1984b) had a tumor rate of 1.5%. While the rates between the two control groups are different, the standard deviation of the historical control rates as indicated in Haseman et al. (1985) is 4.8%, and the range of these rates is 0–10%. Because the concurrent control rate fell within the historical range, this rate was determined to be reasonable, and using concurrent control is generally preferred to using historical control. Therefore, the concurrent control rate was determined to be appropriate for the tumor modeling for RDXFor presentation purposes, the liver and lung tumor data in Lish et al. (1984b) were remodeled using historical control. The data used for these analyses are presented in Table D-26. Note that these data are the same as in Table D-13, except that the percentages were entered into BMDS as the response and the control response for each tumor was set equal to the historical control in Haseman et al. (1985).

Table D-26. Tumor data used for dose-response modeling with historical control

Endpoint and reference	Species/sex	Dose (mg/kg-d)[a]	Total	Percentage with tumors
Hepatocellular adenomas or carcinomas Parker et al. (2006)	B6C3F$_1$ mouse/female	0 1.5 7 35	67[b] 62 63 64	8.3%[c] 6.5% 7.9% 15.6%
Alveolar/bronchiolar adenomas or carcinomas Lish et al. (1984b)	B6C3F$_1$ mouse/female	0 1.5 7 35	65 62 64 64	6.9% 4.8% 12.5% 18.8%

PWG = Pathology Working Group.

[a]The highest dose group was excluded from the analysis because approximately half the animals in the group died from overdosing, which possibly introduces bias in the estimate of response rate in that group. See Section 2.3.1 for more details.

[b]For female mouse hepatocellular tumors from Lish et al. (1984b), tumor incidence and totals are those reported in the PWG re-evaluation (Parker et al., 2006).

[c]The percentages for the control group for both liver and lung tumors were obtained from Haseman et al. (1985); the percentage for each positive dose group for each tumor was calculated by dividing the incidence in that group by the total number of animals in the group.

The BMD model results are provided in Tables D-27 and D-28 (and Figures D-20 and D-21), along with the corresponding plots and model outputs. A comparison of the BMD results is provided in Table D-29. Use of historical control data from NTP (Haseman et al., 1985) had a relatively small impact on the POD used for deriving the OSF. BMD_{10} values for liver and lung tumors using concurrent control data and excluding the high dose from the analysis (i.e., the basis for the OSF in the current assessment) were 25.5 and 29.9 mg/kg-day, respectively. Replacing the incidence of tumors in concurrent controls with NTP historical control data yielded BMD_{10} values for liver and lung tumors of 37.5 and 24.1 mg/kg-day, respectively.

Table D-27. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), using historical control and with highest dose dropped; 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°**	**0.520**	**171.92**	**24.1**	**13.2**	All of the models reduced to the Multistage 1° model, so this model was selected.

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were 0.10, −0.73, 0.85, −0.21, respectively.
[b]For the Multistage 2° and 3° models, the b2 and b3 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1° model.

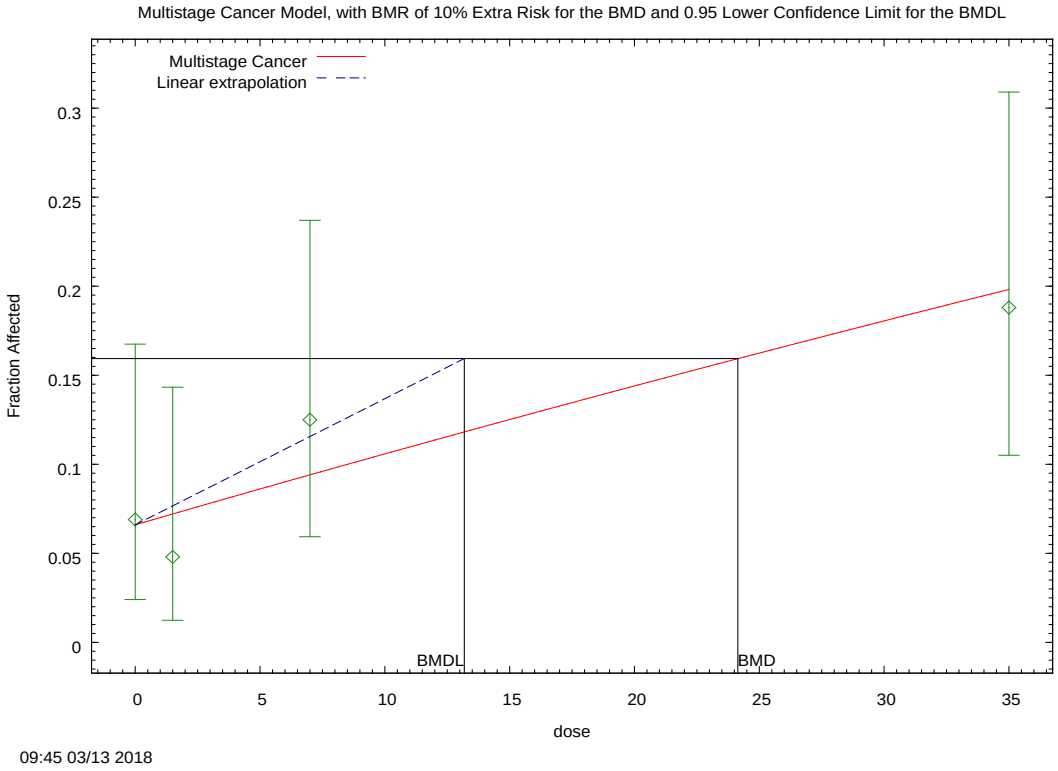


Figure D-20. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 1° model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Lish et al., 1984b), using historical control and with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 24.1402

BMDL at the 95% confidence level = 13.1829

BMDU at the 95% confidence level = 75.0865

Taken together, (13.1829, 75.0865) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00758556

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0659699	0.0688823
Beta(1)	0.00436453	0.00406884

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−83.3032	4			
Fitted model	−83.9602	2	1.31395	2	0.5184
Reduced model	−87.1906	1	7.77481	3	0.0509

AIC = 171.92

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0660	4.288	4.485	65	0.098
1.5	0.0721	4.468	2.976	62	−0.733
7	0.0941	6.021	8.000	64	0.847
35	0.1983	12.690	12.032	64	−0.206

$\chi^2 = 1.31$ d.f. = 2 p-value = 0.5201

Table D-28. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), using historical control and with highest dose dropped; 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°	0.926	162.69	39.9	18.4	The Multistage 2° model had the lowest AIC, so this model was selected.
Multistage 2°[b] Multistage 3°	**0.926**	**162.55**	**37.5**	**18.7**	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, and 35 mg/kg-d were 0.26, −0.30, 0.03, −0.00, respectively.
[b]For the Multistage 3° model, the b3 coefficient estimate was 0 (boundary of parameter space). This model reduced to the Multistage 2° model.

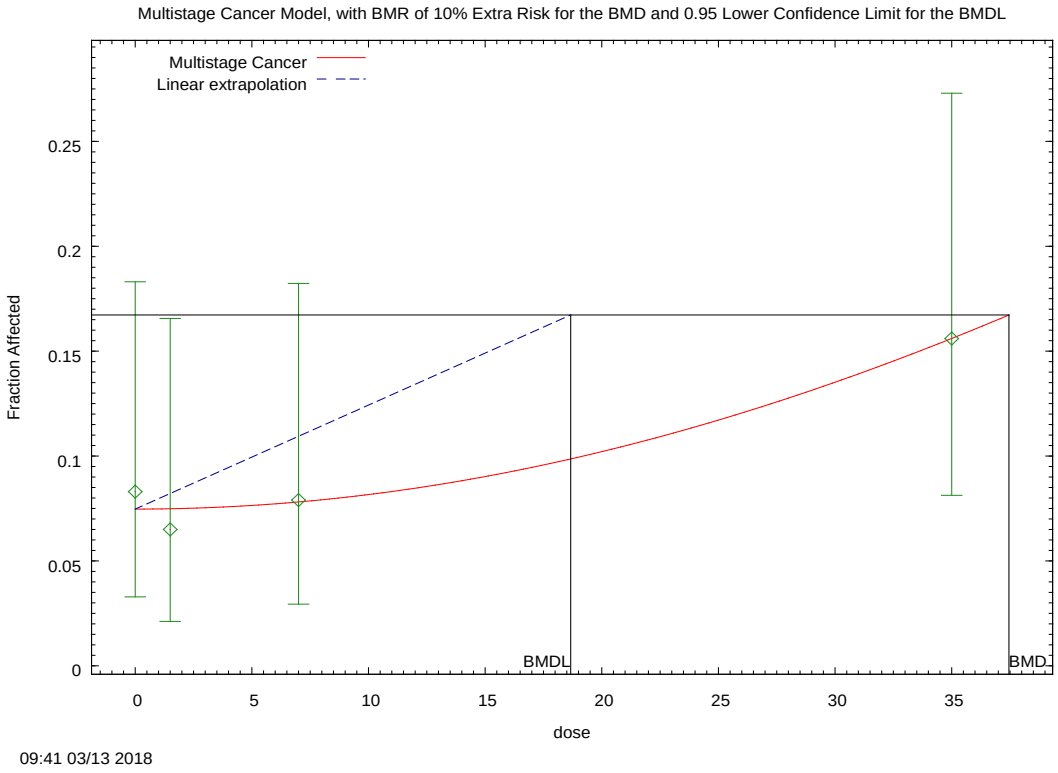


Figure D-21. Plot of incidence rate by dose, with fitted curve for Multistage-Cancer 1° model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), using historical control and with highest dose dropped; BMR = 10% ER; dose shown in mg/kg-day.

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 = 37.4534

BMDL at the 95% confidence level = 18.6687

BMDU at the 95% confidence level = 480758

Taken together, (18.6687, 480758) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00535657

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0.0746974	0.0745007
Beta(1)	0	0
Beta(2)	7.51096E-005	7.52728e-005

Analysis-of-Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test d.f.	*p*-value
Full model	−79.1947	4			
Fitted model	−79.2727	2	0.156066	2	0.9249
Reduced model	−80.8944	1	3.39949	3	0.334

AIC = 162.545

Goodness-of-Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0747	5.005	5.561	67	0.258
1.5	0.0749	4.641	4.030	62	−0.295
7	0.0781	4.920	4.977	63	0.027
35	0.1560	9.986	9.984	64	−0.001

$\chi^2 = 0.15$ d.f. = 2 p-value = 0.9257

Combined results for presence of hepatocellular or alveolar/bronchiolar tumors in B6C3F$_1$ female mice exposed to RDX by diet for 24 months, using historical control and with highest dose dropped; BMR = 10% ER

BMD = 18.3 mg/kg-day; BMDL = 4.86 mg/kg-day

MS-COMBO Results

BMR of 10% Extra Risk

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

  Combined Log-Likelihood                    -163.23289572215137

  Combined Log-likelihood Constant            148.36798304322497


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        18.3472

            BMDL =         4.8583

Multistage Cancer Slope Factor =     0.0205833
```

Table D-29. Comparison of model predictions, for lung and liver tumors in female B6C3F$_1$ mice exposed to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by diet for 24 months (Parker et al., 2006; Lish et al., 1984b), using concurrent and historical control incidence (with highest dose dropped); BMR = 10% ER

Control	Tumor type	BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)
Concurrent	Hepatocellular adenomas or carcinomas	25.5	14.2
	Alveolar/bronchiolar adenomas or carcinomas	29.9	14.9
	Liver + lung	13.8	8.53
Historical[a]	Hepatocellular adenomas or carcinomas	37.5	18.7
	Alveolar/bronchiolar adenomas or carcinomas	24.1	13.2
	Liver + lung	18.3	4.86

[a]Historical control data from NTP (Haseman et al., 1985).

APPENDIX E. SUMMARY OF SCIENCE ADVISORY BOARD (SAB) PEER REVIEW COMMENTS AND EPA'S DISPOSITION

The *Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)*, dated September 2016, underwent a formal external peer review in accordance with U.S. Environmental Protection Agency (EPA) guidance on peer review (U.S. EPA, 2015). This peer review was conducted by the Chemical Assessment Advisory Committee (CAAC) Augmented for Review of the Draft IRIS RDX Assessment (SAB-CAAC RDX panel) of EPA's Science Advisory Board (SAB). An external peer review workshop was held on December 12−14, 2016. Public teleconferences of the SAB-CAAC RDX panel were held on November 17, 2016 and April 13 and 17, 2017. The SAB held a public meeting on August 29, 2017 to conduct a quality review of the draft peer review report. The final report of the SAB was released on September 27, 2017.

The SAB was tasked with providing feedback in response to charge questions that addressed scientific issues related to the hazard identification and dose-response assessment of RDX. Key recommendations of the SAB and EPA's responses to these recommendations, organized by charge question, follow.

1. Literature Search/Study Selection and Evaluation

Charge Question 1. The section on *Literature Search Strategy | Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Key Recommendation: EPA should include a literature search on the role of GABAergic systems in brain development, and how this knowledge can inform a better understanding of the potential developmental neurotoxicity of RDX.

Response: Along with review of references provided by the SAB in the peer review report, a targeted literature search was performed in PubMed to identify literature to supplement discussion in the assessment on the generalized role of GABAergic systems in brain development. As a result of this review, additional language on the role of GABAergic signaling has been added as part of a significantly expanded discussion on potential developmental neurotoxicity of RDX in Section 1.3.3

of the assessment (Susceptible Populations and Life Stages for Cancer and Noncancer Outcomes). Additional language on developmental neurotoxicity has also been included in the discussions of nervous system hazard (1.2.1), derivation of candidate values (2.1.3), and uncertainties in the derivation of the reference dose (2.1.7).

Key Recommendation: EPA should not exclude nonmammalian species as they may bring important mechanistic insight into the draft assessment.

Response: The Literature Search and Screening Strategy section was revised to clarify that studies in nonmammalian and other ecological species were not excluded from the RDX assessment and to recognize the potential use of such studies. Along with revisions to text in the assessment, Figure LS-1 and Table LS-1 were revised to indicate more clearly that nonmammalian/ecological studies were tracked as supplementary studies and used as a source of information to inform the assessment of RDX health effects and potential modes of action.

Key Recommendation: EPA should clarify its reasoning and approach for including or excluding nonmammalian species studies and secondary references.

Response: The Literature Search and Screening Strategy section, including text (see Figure LS-1 and Table LS-1), was revised to clarify that studies in nonmammalian/ecological species were tracked as supplementary information. Secondary references (e.g., regulatory documents, reviews, risk assessments) were excluded as primary sources of health effects data, but were tracked and considered as sources of information in assessing the health effects of RDX.

2. Toxicokinetic Modeling

Charge Question 2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

Key Recommendation: None provided.

Response: No response required.

Charge Question 2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was

not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

Key Recommendation: While current approaches for dose metrics are generally appropriate, the basis for the choice of dose metric for the prostatitis endpoint should be described.

Response: The basis for selecting area under the curve (AUC) for RDX concentration in arterial blood as the internal dose metric for analyzing effects on the prostate, as well as for analyzing effects on the kidney and bladder, was added to Section 2.1.2, Methods of Analysis. Tissue-specific dose metrics for these organs were not available in the PBPK model. AUC was selected as the dose metric because effects on the prostate, kidney, and bladder were observed only after subchronic or chronic exposure to RDX (i.e., there is no evidence that effects were associated with peak exposure) and because greater confidence was placed in model estimates of blood AUC.

Charge Question 2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

Key Recommendation: None provided.

Response: In its review of the draft RDX assessment (SAB, 2017), the SAB expressed agreement with the uncertainty factor (UF) of 10 to account for human variation uncertainty factor (UF_H). Accordingly, no recommendations for use of an alternative UF were offered. Consistent with the position of the SAB, no changes to the UF_H were made.

3. Hazard Identification and Dose–Response Assessment
3.1. Nervous System Effects

Charge Question 3a(i). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

Key Recommendation: Lack of studies on neurodevelopmental toxicity, as well as cognitive and behavioral effects of RDX, should be recognized in the assessment (see discussion in Section 3.3.1.4, Database Uncertainty Factor [UF_D]).

Response: Consistent with the response to Charge Question 1, the revised assessment has an expanded treatment on the lack of developmental neurotoxicity, and notes the general paucity of

information on subconvulsive and/or cognitive and behavioral effects that could be associated with exposure to RDX. These observations have been integrated into multiple parts of the assessment, as relevant, including: Nervous System Effects (1.2.1), Integration and Evaluation of Effects Other than Cancer (1.3.1), Susceptible Populations and Life Stages for Cancer and Noncancer Outcomes (1.3.3), Derivation of Candidate Values (2.1.3), and Uncertainties in the Derivation of the Reference Dose (2.1.7).

Charge Question 3a(ii). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Cholakis et al. (1980) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

Key Recommendation: The problem maintaining uniform dose suspensions should be identified in the EPA assessment as a critical study limitation that increases uncertainty in deriving the RfD based on the Cholakis et al. (1980) study.

Response: A thorough evaluation of methods used to prepare test diets and suspensions was performed for all repeated-dose studies of RDX, focusing on the variability in actual concentrations of the test material and the extent to which target (nominal) concentrations were achieved. The results of this evaluation were documented in Experimental Animal Studies/Exposure under the section Literature Search Strategy | Study Selection and Evaluation. The new text included discussion of issues with maintaining uniform dose suspensions in the Cholakis et al. (1980) study. In addition, Section 2.1.4, Derivation of Organ/System-Specific Reference Doses was revised to include discussion of the highly variable concentrations of RDX in the dose suspensions in Cholakis et al. (1980) as one of the factors considered in selecting the Crouse et al. (2006) study as the basis for the nervous system reference value.

Charge Question 3a(iii). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

Key Recommendation: EPA should consider using a benchmark response (BMR) of 5% for their dose-response modeling of the Crouse et al. (2006) data, while addressing the uncertainty of using data on a frank effect (convulsions in this case) as the basis of an RfD with a larger database uncertainty factor.

Response: Consistent with the recommendations of the SAB, the BMR used for dose-response modeling of convulsion incidence data from both the Crouse et al. (2006) and Cholakis et al. (1980) studies was changed from 1 to 5% ER. The uncertainty associated with using data on a frank effect (convulsions) in the absence of adequate investigation of the potential for RDX to induce subclinical cognitive and behavioral neurological effects was addressed with the application of a larger database uncertainty factor. Section 1.2.1, Methods of Analysis/Nervous System Effects, was revised by adding a discussion of the considerations involved in selecting both a 1 and 5% ER BMR, and the justification for the final selection of a 5% BMR. Appendix D, Tables D-3 and D-4, were revised to present results for the benchmark dose (BMD) analysis based on a 5% ER.

Key Recommendation: If EPA decides to use a BMR of 1% for the dose-response assessment using Crouse et al. (2006), EPA should justify why the greater conservatism in risk assessment required for a frank effect (due to the lack of incidence data for less severe endpoints) is better dealt with through a lower BMR than through application of UF_D.

Response: As noted above, the BMR was revised to 5% ER consistent with the recommendations of the SAB.

Key Recommendation: If EPA decides to use a BMR of 1% for Crouse et al. (2006), EPA should provide clear justification for why a 1% BMR is more appropriate than a 5% BMR for RDX, given the greater uncertainty introduced into the dose-response assessment for RDX using a BMR of 1%.

Response: As noted above, the BMR was revised to 5% ER consistent with the recommendations of the SAB.

Charge Question 3a(iv). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.

Key Recommendation: Consistent with EPA guidance for UFs, the SAB strongly suggests applying the full default UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence

data for less severe neurological effects resulting in use of a severe effect (convulsions) as a basis for the RfD, and the proximity of lethal doses to convulsive doses.

Response: Consistent with the recommendation by the SAB, the revised assessment of RDX applies a database deficiencies uncertainty factor (UF_D) of 10, recognizing significant gaps in the available information to evaluate RDX neurotoxicity. Clarifying text emphasizing the lack of information on subconvulsive effects of RDX exposure has been added throughout relevant sections of the assessment, most notably in the discussion on susceptible populations and life stages (1.3.3), and the rationale for selecting the UF_D. Additionally, clarifying language was added to the examples of information that would reduce database uncertainty to emphasize the need for additional data that informs subconvulsive and other cognitive and behavioral endpoints.

Key Recommendation: EPA should discuss whether potential neurodevelopmental effects of RDX would be sufficiently addressed by the default UF_D of 10, given that the mechanism of RDX argues there would likely be developmental neurotoxic effects and the other database uncertainties (lethality at convulsive doses, other less severe neurotoxic effects that may have a lower LOAEL) that also need to be addressed by the UF_D.

Response: EPA raised the UF_D from 3 to 10, recognizing that the database for RDX contains important gaps in the evaluation of neurotoxic effects, including a lack of developmental neurotoxicity studies as well as studies on subconvulsive and/or cognitive or behavioral effects. Additionally, text was added to the assessment (see Section 1.3.3) describing how bicuculline, a $GABA_A$ receptor antagonist with a similar mode of action to RDX, caused developmental and behavioral impairment in neonatal mice at a dose 1/10 the dose that provoked seizures in adult mice; in the absence of any RDX-specific information, this observation is consistent with application of a UF_D of 10. The UF_H accounts for variation in susceptibility in the human population (including life-stage variability). A UF_H of 10 was also applied in this assessment to account for the possibility that the RDX database does not allow for a full characterization of the exposure-response relationship in the most sensitive portions of the human population [for further discussion on the application of UF_H, please see U.S. EPA (2002)]. EPA concluded that the application of a UF_D of 10 along with a UF_H of 10 sufficiently addressed the uncertainty.

Key Recommendation: SAB recommends that EPA reconsider the UF for subchronic-to-chronic extrapolation (UF_S), and at a minimum, provide stronger justification for a UF_S of 1.

Response: The discussion of the UF_S in Section 2.1.3 was revised to consider support for both a UF_S of 1 and 3 when applied to data from gavage studies of less-than-chronic duration. A UF_S of 1 was retained based on observations that convulsions occurred shortly after dosing (minutes to hours) across most studies and generally did not appear to be influenced by duration of exposure.

Charge Question 3a(v). Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

Key Recommendation: EPA should justify the rationale for utilizing the dose-response data of Crouse et al. (2006) in preference to Cholakis et al. (1980) as the primary basis for the RfD.

Response: As discussed in response to Charge Question 3a(ii), a discussion of deficiencies in the Cholakis et al. (1980) developmental toxicity study introduced by the highly variable concentrations of RDX in dose suspensions was added to the Literature Search Strategy | Study Selection and Evaluation section (under Experimental Animal Studies/Exposure) and to Section 2.1.4, Derivation of Organ/System-Specific Reference Doses. The uncertainties associated with the administered gavage doses in the Cholakis et al. (1980) study were added as further justification for preferring dose-response data from Crouse et al. (2006) over data from Cholakis et al. (1980) as the basis for the nervous system reference value.

Key Recommendation: The SAB recommends increasing the UF_D from 3 to 10.

Response: As discussed in response to Charge Question 3a(iv), the UF_D in the revised assessment was increased from 3 to 10.

Key Recommendation: The SAB recommends revisiting the UF_S and providing a better justification, at a minimum, for the use of a UF_S of 1.

Response: As discussed in response to Charge Question 3a(iv), justification of the UF_S was revised to better support the value of 1 for this uncertainty factor.

3.2. Kidney and Other Urogenital System Effects

Charge Question 3b(i). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

Key Recommendation: Suppurative prostatitis should not be used as a surrogate marker of renal and urogenital effects, and instead, be considered a separate hazard of RDX exposure (see also Section 3.3.2.5.) for quantitative risk assessment.

Response: As recommended by the SAB, the incidence of suppurative prostatitis was removed from discussion on urinary system effects, and instead moved to a new section of the Hazard identification, Prostate Effects (1.2.3). The revised discussion on prostate effects was expanded to discuss prostate-specific findings and the broader continuum of inflammation. Consistent with SAB recommendations, the incidence of suppurative prostatitis was selected as the endpoint most representative of prostate effects. A prostate-specific reference dose was derived based on this endpoint as well.

Key Recommendation: The description and analysis of prostatitis should be expanded to include discussion of both chronic and suppurative inflammation.

Response: Text was added to the new prostate effects section (see Section 1.2.3) to discuss both chronic and suppurative inflammation. A new table was added to the assessment identifying the incidence of all types of prostate inflammation in Levine et al. (1983).

Key Recommendation: The description of the various uncertainties regarding the Levine et al. (1983) rat study should be expanded to include commentary on lack of detail on methods used in histopathological evaluations, lack of peer review, and the impact of the high prevalence of fighting in highest dose rats.

Response: Additional information on potential differences in histopathology methods and the potential impact of fighting was included in the Prostate Effects section (see Section 1.2.3), as well as discussion on the selection of the overall reference dose (see Section 2.1.5). It should be noted that as part of the assessment development process, EPA had the 2-year bioassays by Levine et al. (1983) and Lish et al. (1984b), the subchronic toxicity study by Crouse et al. (2006), and the collection of repeated-dose studies reported in (Cholakis et al., 1980) peer reviewed. The results of these peer reviews are discussed in the Study Evaluation section of the assessment and are available at https://hero.epa.gov/hero/.

Charge Question 3b(ii). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

Key Recommendation: Improve the discussion and analysis of renal effects observed in studies other than those reported by Levine et al. (1983).

Response: Synthesis of the evidence for kidney and urinary bladder effects associated with RDX exposure in experimental animals was substantially revised. The section title was changed to "Urinary System (Kidney and Urinary Bladder) Effects" to reflect the revised focus of the section, and more detailed findings from the subchronic studies were added. The evidence table pertaining

to urinary system effects (see Table 1-5) was revised by including additional histopathological findings from the 13-week studies by Levine et al. (1981a) and Martin and Hart (1974). The exposure-response array (see Figure 1-2) was revised to be consistent with changes made to the evidence table.

Key Recommendation: Include a brief discussion of the marked sex difference in the renal toxicity in rats due to RDX exposure.

Response: Discussion of the marked sex difference in kidney response to RDX based on the Levine et al. (1983) study was added to the synthesis text in Section 1.2.2 and to the integration of evidence for urinary system effects.

Charge Question 3b(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

Key Recommendation: The SAB strongly recommends that suppurative prostatitis should be used as a stand-alone endpoint, separate from kidney and other urogenital system endpoints for calculation of the POD and HED.

Response: Consistent with the SAB's recommendation, EPA revised the assessment to develop a separate evaluation of prostate effects (see Section 1.2.3). Suppurative prostatitis was identified as the endpoint most representative of prostate effects and for calculating the related POD and human equivalent dose (HED).

Charge Question 3b(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Key Recommendation: Develop or cite documentation for the use of organ-specific reference values for individual chemicals, including how these would be used in assessing the combined noncancer health impacts of multiple agents acting at a common site in cumulative risk assessments.

Response: Section 2.1.4 was expanded to provide examples of the use of organ/system-specific reference values in cumulative risk assessments, including how these values can be used to refine the hazard index approach described in EPA's *Risk Assessment Guidance for Superfund* (U.S. EPA, 1989).

Key Recommendation: A separate RfD should be derived for renal papillary necrosis and the associated renal inflammation of the kidney and urogenital system.

Response: An organ/system-specific oral reference dose (RfD) for effects on the urinary system (kidney and urinary bladder) was derived separate from one for prostate effects. Section 1.3.1 and Chapter 2 were revised accordingly to characterize effects of RDX on the prostate as separate from the effects on the kidney and urinary bladder. As described in Sections 2.1.1 to 2.1.4, dose-response analyses were conducted for kidney (medullary papillary necrosis) and urinary bladder (hemorrhagic/suppurative cystitis) data sets as reported by Levine et al. (1983). A no-observed-adverse-effect level (NOAEL) was used as the POD for incidence of medullary papillary necrosis in the kidney because this data set was considered unsuitable for BMD modeling; incidence data for hemorrhagic/suppurative cystitis in the urinary bladder were amenable to BMD modeling. Because the PODs derived from these two data sets were similar, a single organ/system-specific RfD for the urinary system was developed.

Key Recommendation: The male accessory sex glands should be designated as a separate organ system with a separate RfD derived for suppurative prostatitis.

Response: A new section was developed in the hazard identification (see Section 1.2.3) that specifically evaluated the available data to characterize effects of RDX on the prostate. As described in revised text in Section 1.3.1, EPA determined that the incidence of suppurative prostatitis was the endpoint most appropriate for representing prostate effects. The Levine et al. (1983) study was selected for dose-response analysis, as the only study to identify an increased incidence of suppurative prostatitis (see Sections 2.1.1 to 2.1.4). Incidence data were amenable to BMD modeling with a BMR of 10% extra risk, and an organ/system-specific RfD for prostate effects was derived.

Charge Question 3b(v). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

Key Recommendation: Separate RfDs should be derived for renal papillary necrosis and the associated renal inflammation and for suppurative prostatitis.

Response: As discussed in response to Charge Question 3b(iv), separate organ/system-specific RfDs were derived for the urinary system (based on medullary papillary necrosis of the kidney and hemorrhagic/suppurative cystitis of the urinary bladder) and prostate (based on suppurative prostatitis). Section 1.3.1 and Chapter 2 were revised accordingly.

Key Recommendation: Available data are not consistent enough across species, doses, sex, or time points to recommend separate candidate RfDs for other, milder renal effects (tubular nephrosis,

epithelial vacuolization, and mineralization) found in subchronic studies in mice, rats, and monkeys.

Response: EPA agrees that evidence for the milder renal effects found in subchronic studies of RDX in mice, rats, and monkeys was inconsistent. Further, findings from the subchronic studies that provided some evidence of renal effects were difficult to interpret. For example, positive findings were observed only at a very high dose (320 mg/kg-day) in mice, minimal to mild mineralization of the medulla in monkeys was not recognized as treatment related by the study authors, and renal tubular epithelial-lined cysts in the kidney cortex were reported in a study that performed histopathology only for F2-generation rats, with exposure to RDX limited to gestation and weaning. Discussion of the findings from the subchronic studies in Section 1.2.2, Urinary System (Kidney and Urinary Bladder) Effects, was revised to better characterize the nature of these findings. In addition, the discussion of kidney and urinary bladder effects in Section 1.3.1 (Effects Other Than Cancer) was revised to include characterization of subchronic findings as limited and inconsistent. EPA agrees that the renal findings from subchronic studies do not support derivation of a candidate reference value. Only kidney and urinary bladder data from the 2-year bioassay by Levine et al. (1983) were used for dose-response analysis.

3.3. Developmental and Reproductive System Effects

Charge Question 3c(i). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

Key Recommendation: Due to significant weaknesses of the findings in the rat and mouse studies, RDX should not be considered as having suggestive evidence of male reproductive effects.

Response: The section on male reproductive effects was revised to be consistent with recommendations from the SAB. In re-evaluating the available evidence, EPA concurred with the SAB's determination that the available evidence did not support an association between RDX exposure and male reproductive effects. The hazard determination for this hazard was revised to "there is insufficient information to assess male reproductive toxicity following exposure to RDX." In light of the revised hazard determination, discussion of male reproductive effects was moved to Section C.3.2, Other Noncancer Effects, in the Supplemental Information. A summary of the evidence for male reproductive effects was included in Section 1.2.6 of the Toxicological Review

with other noncancer effects for which there is inadequate information to assess an association with RDX exposure.

Charge Question 3c(ii). Is the selection of the Lish et al. (1984b) study that describes male reproductive system effects scientifically supported and clearly described?

Key Recommendation: SAB finds that derivation of a reproductive-system specific toxicity value is not justified, as there have been no convincing studies showing significant male reproductive toxicity.

Response: The Toxicological Review was revised to remove derivation of an organ/system-specific RfD for male reproductive effects from Chapter 2.

Charge Question 3c(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

Key Recommendation: No POD for reproductive endpoints should be calculated from the existing data and therefore there is no need to calculate the HED.

Response: As noted in response to Charge Question 3c(ii), the Toxicological Review was revised to remove derivation of an organ/system-specific RfD for male reproductive effects from Chapter 2, including calculation of a POD and HED.

Charge Question 3c(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

Key Recommendation: Since no valid POD should be calculated for reproductive endpoints, there is no need to discuss UFs for reproductive endpoints.

Response: No response required.

Charge Question 3c(v). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

Key Recommendation: No RfD based on male reproductive toxicity should be calculated since no valid POD can be estimated.

Response: As noted in response to Charge Question 3c(ii), the Toxicological Review was revised to remove derivation of an organ/system-specific RfD for male reproductive effects from Chapter 2.

3.4. Other Noncancer Hazards

Charge Question 3d. The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision. Are other noncancer hazards adequately described?

Key Recommendation: For each of the other noncancer hazards discussed in the draft assessment, add a summary statement regarding whether the available studies do, or do not, support a conclusion that the identified toxicity is a potential human hazard. Include an explanation of the rationale for reaching the conclusion, taking into consideration the additional information pertaining to liver effects, the muscle injury, immune system, neuroinflammation and gastrointestinal effects.

Response: In Section C.3.2 in the Supplemental Information, a statement was added for each organ/system, based on the synthesis and integration of evidence from human and animal studies, as to whether the available health effects information was adequate to support a hazard determination. Additionally, other organ/systems were included during revision, and information was added where warranted (e.g., neuroinflammation). Consistent across all endpoints included in Other Noncancer Effects, there is insufficient information to assess toxicity following exposure to RDX. The rationale for these conclusions, taking into consideration consistency of the findings within and across studies, biological significance of the findings, and study confidence, was provided with each organ/system-specific discussion.

Key Recommendation: Include as a potential noncancer hazard the available subchronic and chronic data on body weight/body-weight gain, and whether the studies do, or do not, support a conclusion that body-weight effects represent a potential systemic human hazard. Discuss the rationale for the conclusion and explain why body-weight effects are, or are not, carried forward to the dose-response analysis.

Response: Terminal body-weight data from subchronic and chronic studies of RDX were summarized in an evidence table (see Table C-18), and a synthesis of the evidence for body-weight changes associated with RDX exposure was added to Section C.3.2, Other Noncancer Effects, in the Supplemental Information. In general, biologically significant decreases in body-weight gain (considered to be a decrease of ~10% relative to controls) were observed at ≥40 mg/kg-day—RDX doses that also produced severe toxicity in animals (e.g., serious kidney toxicity in male rats and lethality in other studies). At lower doses, no apparent pattern of treatment-related body-weight

change within or across studies was observed. Because decreased body-weight gain appears to be secondary to other primary targets of toxicity that were brought forward for dose-response analysis, change in body weight was not considered a systemic hazard in and of itself and therefore was not carried forward for dose-response analysis.

3.5. Cancer

Charge Question 3e(i). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

Key Recommendation: Strengthen and make more specific the justification for selecting the "*suggestive evidence of carcinogenic potential*" descriptor rather than the "*likely to be carcinogenic to humans*" descriptor.

Response: The justification for selecting the cancer descriptor of "*suggestive evidence of carcinogenic potential*" was strengthened by adding more detailed information on the individual cancer bioassays to both Section 1.2.7 (synthesis of evidence for carcinogenicity) and Section 1.3.2 (cancer descriptor). Specifically, the revised text included additional discussion of the mortality time course observed in the bioassays in mice (Lish et al., 1984b) and rats (Levine et al., 1983) (see Section 1.2.7); clarification of the evidence for rat liver tumors (see Section 1.2.7); summary of concerns raised by the Pathology Working Group (PWG) regarding female mouse liver tumors and observations regarding the low liver tumor incidence in female controls (see Sections 1.2.7 and 1.3.2); discussion of the evidence for cancer mode of action, including lack of evidence for precursor events leading to liver and lung tumor response (see Section 1.3.2); and lack of a full pathology peer review in all three cancer bioassays of RDX (see Section 1.2.7).

Charge Question 3e(ii). As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984b) study for this purpose scientifically supported and clearly described?

Key Recommendation: None provided.

Response: In its review of the draft RDX assessment (SAB, 2017), the SAB stated that EPA's rationale for quantitative cancer dose-response analysis using the Lish et al. (1984b) study was supported scientifically and clearly described. Accordingly, the SAB offered no recommendations for revising the rationale for undertaking a quantitative analysis. Consistent with the position of the SAB, no changes to this section of the assessment were made.

Charge Question 3e(iii). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

Key Recommendation: For liver cancer, perform and discuss results from additional BMD modeling (i.e., a sensitivity analysis documented in the Supplemental Materials) that examines and discusses:

- The impact of low concurrent controls on model choice and the POD estimate.
- The effect of including/excluding the highest dose has on model choice and the POD estimate.

Response: A sensitivity analysis was conducted and added to Appendix D (see Section D.2.4) to investigate (1) the effect of dropping the highest dose group on model choice and the POD estimate, (2) the impact of low concurrent control liver tumor incidence on model selection and the POD estimate, and (3) the fit of the multistage models in the low-dose region. EPA's more detailed response to item (3) is provided under the next key recommendation.

To examine the effect of including/excluding the highest dose on model choice and the POD estimate, EPA remodeled the female mouse liver and lung tumor data from Lish et al. (1984b) after excluding the highest dose group from the analysis because of the high mortality in that group (i.e., loss of almost half the animals in that group before the dose was dropped from 175 to 100 mg/kg-day at Week 11 of the 2-year study). Excluding the high-dose group from the analysis resulted in a change in the oral slope factor (OSF) from 0.04 to 0.08 $(mg/kg\text{-}day)^{-1}$. In light of the high mortality, EPA determined that it was appropriate to exclude the high-dose group from the analyses because mice that survived the 11-week exposure to 175 mg/kg-day RDX may not have constituted an unbiased representation of the population of animals exposed for the full 2-year study (i.e., those more or less sensitive to RDX than the animals in the general population). The rationale for dropping the high-dose group from the data set used for dose-response modeling was provided in Section 2.3.1, and the documentation of the modeling in Appendix D was revised to present the reanalysis with the high-dose group dropped.

To address the impact of low concurrent control liver tumor incidence on model selection and the POD, EPA conducted a dose-response analysis of female mouse liver and lung tumor data using historical control data from National Toxicology Program [NTP; (Haseman et al., 1985)]. This analysis revealed that historical control incidence had a relatively small impact on the POD (i.e., $BMDL_{10}$) used for deriving the OSF. $BMDL_{10}$ values for liver and lung tumors using concurrent control data and excluding the high dose from the analysis were 14.2 and 14.9 mg/kg-day, respectively. Replacing the incidence of tumors in concurrent controls with NTP historical control data yielded $BMDL_{10}$ values for liver and lung tumors of 18.7 and 13.2 mg/kg-day, respectively. EPA notes a preference for using concurrent control data, especially as in the current case where historical control data originated from a different laboratory where there was potential for cross-study differences in diet, laboratory, pathological evaluation, and animal provider. The analysis using historical control data from NTP is presented as part of the sensitivity analysis added to Appendix D, Section D.2.4; however, EPA retained the analysis using concurrent control tumor data as the basis for the OSF.

Key Recommendation: Provide details and discuss the adequacy of the fit of the multistage model to available data.

Response: The adequacy of the fit of the multistage model to the female mouse liver and lung tumor data was addressed in the context of the sensitivity analysis added to Appendix D, Section D.2.4. As discussed in Section D.2.4, EPA agreed with the SAB that the multistage models exhibited an inferior fit to the female mouse liver tumor data with all doses included compared to a subset of nonmultistage models in benchmark dose software (BMDS); however, with the high-dose group dropped, the multistage models yielded a fit comparable to the best-fitting model. For female mouse lung tumors, the multistage models exhibited fits comparable to the best-fitting model among multistage and nonmultistage models both with all doses included and with the high-dose group dropped.

Key Recommendation: Provide a better and more detailed description of the MS-COMBO methodology (in the Supplemental Information document) and ensure that this description discusses the issues below:

- importance of the assumption of independence,
- why this assumption is needed,
- how this assumption might be examined statistically given adequate study data/documentation,

- whether the independence of tumor incidence assumption further constrains the tumor-specific dose-response model form to be the same across included tumor types, and,
- the extent to which violations of the independence of tumor incidence assumption negatively affect the estimated POD.

Response: A more detailed and comprehensive description of the MS-COMBO methodology was added to Appendix D, Section D.2.1; reference to this description of MS-COMBO was added to Section 2.3.2 of the Toxicological Review. In particular, the importance and testability of the assumption of independence was discussed in greater detail.

4. Dose-Response Analysis

Charge Question 4a. The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006). Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

Key Recommendation: The SAB agrees that the overall RfD should be based on neurotoxicity as measured by convulsions in Crouse et al. (2006), but the SAB concludes that the scientific support for the proposed oral RfD is somewhat lacking primarily due to concerns with the choice of BMR and the value of the database uncertainty factor and the uncertainty factor for subchronic-to-chronic extrapolation. This deficiency needs to be rectified.

Response: As noted in responses to Charge Questions 3a(iii) and 3a(iv), EPA revised the choice of the BMR from 1% to 5% extra risk and the UF_D from 3 to 10, consistent with the recommendations of the SAB. The rationales supporting the revised BMR and UF_D are provided in the toxicological review (see Sections 2.1.2 and 2.1.3). In addition, EPA provided stronger justification for the uncertainty factor for UF_S of 1 (see Section 2.1.3).

Key Recommendation: Since the Cholakis et al. (1980) study suffers from several quality issues, it is appropriate to give more weight to the Crouse et al. (2006) study with respect to the quantitative dose-response analysis. The rationale for the selection of Crouse et al. (2006) and setting aside the Cholakis et al. (1980) study even though it reported a lower NOAEL/LOAEL, should be strengthened and clarified.

Response: As noted in responses to Charge Questions 3a(ii) and 3a(v), a discussion of deficiencies in the Cholakis et al. (1980) developmental toxicity study introduced by the highly variable

concentrations of RDX in dose suspensions was added to the Literature Search Strategy | Study Selection and Evaluation section (under Experimental Animal Studies/Exposure). Section 2.1.4, Derivation of Organ/System-Specific Reference Doses, was revised to provide further discussion of the deficiencies in the Cholakis et al. (1980) study and a stronger justification for using dose-response data from Crouse et al. (2006) [over data from Cholakis et al. (1980)] as the basis for the nervous system reference value.

Key Recommendation: The discussion and key recommendations from Section 3.3.1.3 and Section 3.3.1.4 (*of the SAB report*) are all pertinent to the SAB finding that the scientific support for, and discussion of, the proposed oral RfD for the convulsions endpoint is lacking. These recommendations are repeated here:

- EPA should consider using a BMR of 5% for their dose-response modeling of the Crouse et al. (2006) data while addressing the uncertainty of using data on a frank effect (convulsions in this case) as the basis of an RfD with a larger database uncertainty factor.
- If EPA decides to use a BMR of 1% for the dose-response assessment using Crouse et al. (2006), EPA should justify why the greater conservatism in risk assessment required for a frank effect (due to the lack of incidence data for less severe endpoints) is better dealt with through a lower BMR than through application of UF_D.
- If EPA decides to use a BMR of 1% for Crouse et al. (2006), EPA should provide in its discussion clear justification for why a 1% BMR is more appropriate than a 5% BMR for RDX, given the greater uncertainty introduced into the dose-response assessment for RDX using a BMR of 1%.
- Consistent with EPA guidance for uncertainty factors, the SAB strongly recommends that EPA apply the full default UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe neurological effects resulting in use of a severe effect (convulsions) as a basis for the RfD, and the proximity of lethal doses to convulsive doses.
- EPA should discuss whether potential neurodevelopmental effects of RDX would be sufficiently addressed by a UF_D of 10, given that the mechanism of RDX argues there would likely be developmental neurotoxic effects and the other database uncertainties (lethality at convulsive doses, other less severe neurotoxic effects that may have a lower LOAEL) that also need to be addressed by the UF_D.
- EPA should reconsider the value of the UF_S and at a minimum provide stronger justification for a UF_S of 1.

Response: As discussed in response to Charge Questions 3a(iii) and 3a(iv), consistent with the recommendations of the SAB, the BMR used for dose-response modeling of convulsion incidence data was changed from 1 to 5% ER, and the UF_D was increased from 3 to 10 to account for

significant gaps in neurotoxicity testing for RDX. As discussed in response to Charge Question 3a(iv), the UF_S of 1 was retained, and the justification was revised to better support the value of 1 for this uncertainty factor.

Charge Question 4b. The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

Key Recommendation: EPA should not attempt to derive an inhalation reference concentration since neither toxicokinetic data nor exposure levels information from animal or human RDX inhalation studies are available to make estimation possible.

Response: No response required.

Charge Question 4c. The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

Key Recommendation: Acknowledge that the issues with the estimation of the POD for estimation of the cancer slope factor as discussed in Section 3.3.5.3 (*of the SAB report*) and note that the associated key recommendations for improving the presentation on the POD also apply to the estimation of the cancer slope factor.

Response: As discussed in response to Charge Question 3e(iii), EPA addressed the SAB's concerns regarding the derivation and presentation of the OSF as follows:

- The female mouse liver and lung tumor data from Lish et al. (1984b) were remodeled after excluding the highest dose group from the analysis in light of the high mortality in that group, resulting in a change in the OSF from 0.04 to 0.08 $(mg/kg\text{-}day)^{-1}$.
- A sensitivity analysis was conducted to investigate (1) the fit of the multistage models in the low-dose region, (2) the effect of dropping the highest dose group, and (3) the impact of low concurrent controls on model selection and the POD estimate. This sensitivity analysis was added to Appendix D (see Section D.2.4).

Charge Question 4d. The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of

inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

Key Recommendation: EPA should not attempt to derive an inhalation unit risk since there are no study data available to make estimation possible.

Response: No response required.

5. Executive Summary

Charge Question 5. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Key Recommendation: None provided.

Response: No response required.

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