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

(CASRN 121-82-4)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

Supplemental Information

September 2014

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

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ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
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
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CICADS	Concise International Chemical Assessment Documents
CNS	central nervous system
CSF	cerebrospinal fluid
CYP450	cytochrome P450
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	electroencephalography
EHC	Environmental Health Criteria
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FM	Fort Meade
GI	gastrointestinal
HERO	Health and Environmental Research Online
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
HMX	cyclotetramethylene-tetranitramine
i.p.	intraperitoneal
i.v.	intravenous
IARC	International Agency for Research on Cancer
IH	industrial hygiene
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
LAAP	Louisiana Army Ammunition Plant
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
MRL	Minimal Risk Level
NADPH	nicotinamide adenine dinucleotide phosphate
NAP	National Academies Press
NAS	National Academy of Science
NCE	normochromatic erythrocytes
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NCTR	National Center for Toxicological Research
ND	not detected
NIEHS	National Institute of Environmental Health Sciences
NIOSHTIC	National Institute for Occupational Safety and Health Technical Information Center
NR	not reported
NSCEP	National Service Center for Environmental Publications
NTP	National Toxicology Program
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PND	postnatal day
POD	point of departure
REL	recommended exposure limit
ROD	record of decision
SD	standard deviation
STEL	short-term exposure limit
TLV	threshold limit value
TNT	trinitrotoluene
TNX	1,3,5-trinitroso-1,3,5-triazacyclohexane
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
UF	uncertainty factor
WHO	World Health Organization

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

Table A-1. Assessments by Other National and International Health Agencies

Organization	Toxicity value
Agency for Toxic Substances and Disease Registry (ATSDR, 2012)	Acute oral minimal risk level (MRL)—0.2 mg/kg-d Basis: tremors and convulsions in rats (Crouse et al., 2006); application of a composite uncertainty factor (UF) of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [PBPK modeling] and 10 for human variability) Intermediate oral MRL—0.1 mg/kg-d Basis: convulsions in rats (Crouse et al., 2006); application of a composite UF of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [PBPK modeling] and 10 for human variability) Chronic oral MRL—0.1 mg/kg-d Basis: tremors and convulsions in rats (Levine et al., 1983); application of a composite UF of 30 (3 for extrapolation from animals to humans with dosimetric adjustments [PBPK modeling] and 10 for human variability)

Organization	Toxicity value
American Conference of Governmental Industrial Hygienists (ACGIH, 2011, 2001)	Threshold Limit Value (TLV)—0.5 mg/m^3, time weighted average (TWA) for an 8-hr workday in a 40-hr workweek Basis: Intended to minimize the potential for adverse hepatic, prostate, and hematopoietic effects reported in long-term oral studies in experimental animals. ACGIH documentation does not describe how dose-response data from oral studies was extrapolated to inhalation exposures, or whether other factors were applied to account for extrapolation of animal data to humans. Skin notation indicates potential for systemic exposure and/or toxicity via dermal absorption. Basis: A report of five cases of RDX-exposed munition workers with convulsions and/or loss of consciousness in a plant where mechanical ventilation was absent, material handling was poorly controlled, and rules regarding wearing of respirators and hand-washing were often ignored {Kaplan, 1965, 630095}. Authors identified inhalation, ingestion, and possibly skin absorption as exposure routes; dermal exposure was not specifically documented. {ACGIH, 2001, 630056@@author-year} acknowledged that data related to dermal exposure and toxic effects were very limited, but that "a conservative approach to minimize potential toxic effects warrants inclusion of a skin notation for cyclonite." Class A4 (Not classifiable as a human carcinogen) Basis: Statistically significantly increased incidence of hepatocellular adenomas and carcinomas in B6C3F$_1$ mice {Lish, 1984, 630027@@author-year} were determined to be of little biological significance.
Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS Health Substances Information System Database http://www.nicnas.gov.au/industry/aics/search.asp, accessed May 2, 2012)	Exposure Standard—1.5 mg/m^3 TWA for an 8-hr workday Basis: adopted from the ACGIH TLV established in 1969 Skin absorption notice indicates that absorption through the skin may be a significant source of exposure Basis: adopted from ACGIH
National Institute of Occupational Safety and Health (NIOSH Pocket Guide online http://www.cdc.gov/niosh/npg/default.html, accessed May 2, 2012)	Recommended Exposure Limit (REL)—1.5 mg/m^3 TWA for up to a 10-hr workday during a 40-hr workweek Basis: adopted from the ACGIH TLV established in 1969 Skin designation indicates potential for dermal absorption Basis: adopted from ACGIH
Occupational Safety and Health (OSHA PEL for Maritime and Construction Industries; 29 CFR 1915.1000 Table Z-Shipyards and 29 CFR 1926.55 Appendix A)	Permissible Exposure Limit (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

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

The literature search for RDX was conducted in five online scientific databases; the most recent update was conducted in January, 2014. The detailed search strategy used to search four of these databases—Pubmed, Toxline, Toxcenter, and TSCATS—is provided in Table B-1. The search strategy used to search the Defense Technical Information Center (DTIC) database is described in Table B-2. The computerized database searches were augmented by review of online regulatory sources as well as "forward" and "backward" Web of Science searches of two recent reviews (Table B-3).

Defense Technical Information Center (DTIC) Literature Search and Screen

Eight hundred sixty-seven RDX-related citations were identified in the DTIC database; 510 were the full-text documents with unlimited distribution, 307 were classified as "distribution limited to U.S. Government agencies only," and 50 were classified as "distribution limited to Department of Defense only." Of the 867 citations, eight citations with unlimited distribution and 10 citations with limited distribution were selected for further review. The eight citations with unlimited distribution (that were not duplicated in other databases) were uploaded to the Health and Environmental Research Online (HERO) website[1] (http://hero.epa.gov). The 10 limited-distribution citations were evaluated for pertinence to the health effects of RDX (i.e., with a focus on whether they provided additional primary health effects data) to determine whether 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 citation resulted in the following determinations:

- 4 of the 10 citations were excluded from further consideration because the reports were not specific to RDX, or addressed environmental properties (e.g., leaching);
- 3 of the citations were excluded because they did not provide additional primary health effects data. The citations either described a study plan for, or reported data from, experiments that

[1]HERO is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

were subsequently published (Williams et al., 2011; Hathaway and Buck, 1977) and had already been identified by the literature search strategy;

- 1 citation was identified as actually having unlimited distribution (duplicate record in DTIC database), and was added to the HERO database (Lish et al., 1984);

- 1 citation provided animal inhalation data and was considered pertinent, but was not brought forward for further review because flaws in the design of study were such that results would not be considered credible. These study design issues included lack of a control group, small numbers of animals, incomplete information on dosage or exposure levels, and inadequate reporting;

- 1 citation did not have an abstract or full text available outside of the Department of Defense. Based on the title, this report appeared to deal specifically with the manufacture and chemical/explosive properties of RDX. Given the available information, it was determined that it was unlikely the report would provide primary health effects data that warranted further review.

The rationales for exclusion of the other 849 references that were not selected for further consideration are summarized in Table B-4.

Disposition of Studies Kept for Further Review in July 2013 Preliminary Materials

In EPA's 2013 *Preliminary Materials for the Integrated Risk Information System (IRIS) Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)*, 15 studies were identified as "kept for possible further review," including papers with no abstract, inadequate information in the abstract, or available only in a foreign language. EPA sought input from the public on the utility of these studies in the development of the Toxicological Review. No public comments on the usefulness of these 15 papers to assessing the health effects of RDX were received at the December 2013 bimonthly meeting discussion of the RDX preliminary materials. Upon further review, none of the 15 references were determined to be pertinent to an assessment of the health effects of RDX following chronic exposure or a source of information significantly different from other studies identified through the literature search. Of the 15 citations reviewed:

- 5 of the 15 references described case reports of animal poisoning.

- 5 of the 15 articles were in a foreign language and either due to their age (published before 1960) or title were determined not to provide additional information to substantively inform the toxicity of RDX.

- 2 of the 15 references were not published in the peer-reviewed literature and did not appear to significantly add to other literature sources.

- 1 of the 15 references, based on limited information, appeared to be a site-specific investigation of poisonings that did not substantively address the chronic toxicity of RDX.

- 1 of the 15 references, based on limited information, appeared to be a review/annotated bibliography of other literature and would not significantly add to the literature already identified through the literature search process.

- 1 of the 15 references was a meeting abstract.

Additional References Not Specific to RDX

During assessment development, 93 additional references were cited as sources of information used to help explain or clarify an issue raised in assessing the health effects of RDX, but were not specific to RDX and were not identified through the chemical-specific literature search strategies described in Figure LS-1. Other references cited in the Toxicological Review that are not specific to RDX include EPA guidelines and related documents. These references were tracked in HERO as an additional search strategy ("references added during assessment development"). For transparency, those articles are then automatically included as Secondary Sources of Health Effects Information in the overall literature search strategy.

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

Database	Set #	Terms	Hits
PubMed Date: 4/2012	1A1	((((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	337

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

Database	Set #	Terms	Hits
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PubMed Date limit: 1/2012–2/2013	1A2	(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	1A3	(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 -	138

Database	Set #	Terms	Hits
		MeSH]) OR ("2012/11/01"[Date - Entrez] : "3000"[Date - Entrez]) OR ("2012/11/01"[Date - Create] : "3000"[Date - Create]))	
Toxline Date: 4/2012	1B1	Notes: Searched CASRN or synonyms; -removed invertebrates, aquatic organisms, amphibians, earthworms	507
Toxline Date limit: 2011– 2/2013	1B2	@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+2011+2013+@NOT+@org+pubmed+pubdart+crisp+tscats	5
	1B3	@OR+("1,3,5-Trinitroperhydro-1,3,5-triazine"+"Esaidro-1,3,5-trinitro-1,3,5-triazina"+"Hexahydro-1,3,5-trinitro-1,3,5-triazin"+"Perhydro-1,3,5-trinitro-1,3,5-triazine"+"Cyclotrimethylenenitramine"+"Trimethylenetrinitramine"+"Trimethylene+trinitramine"+"Trimethyleentrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"CX+84A"+"Cyklonit"+"Geksogen"+"Heksogen"+"Hexogeen"+"Hexolite"+"KHP+281")+@AND+@range+yr+2011+2013+@NOT+@org+pubmed+pubdart+crisp+tscats	0
Toxline Date limit: 2012– 1/2014	1B4	@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
	1B5	@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
TSCATS Date: 2/2013	1C1	@term+@rn+121-82-4+@AND+@org+tscats	4
TSCATS Date limit: 2012– 1/2014	1C2	@OR+(@term+@rn+121-82-4)+@AND+@range+yr+2012+2014+@AND+@org+tscats	0

Database	Set #	Terms	Hits
Toxcenter Date: 4/2012	1D1	((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 (20 selected and added to HERO)
Toxcenter Date limit:	1D2	(((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-	26 (6 selected and added to HERO)

Database	Set #	Terms	Hits
1/1/2012–2/2013		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	

Database	Set #	Terms	Hits
Toxcenter Date limit: 11/1/2012–1/2014	1D3	(((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 (0 selected)

1

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

Database	Set #	Terms	Hits
DTIC Online Access Controlled Date: 2/11/2013	2A1	key:((toxicity OR phramacokinetics OR toxicology OR pharmacology OR poisoning OR toxic hazards OR radiation hazards OR radiation effects OR toxic diseases OR toxic agents OR lethal agents OR antidotes OR death OR "signs and symptoms" OR cancer OR carinogens OR physiology OR biochemistry OR body weight OR anatomy OR body fluids OR metabolism OR immunology OR mutagens OR teratogenic compounds OR mutations OR antimetabolites) NOT (aquatic animals OR Invertebrates OR venomous animals OR wildlife OR biodegradation)) and ("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 Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR Hexolite) and distco:(A) | Report Date: All dates	504 (8 selected and added to HERO)
	2A2	distco:(govt) and key:((toxicity OR phramacokinetics OR toxicology OR pharmacology OR poisoning OR toxic hazards OR radiation hazards OR radiation effects OR toxic diseases OR toxic agents OR lethal agents OR antidotes OR death OR "signs and symptoms" OR cancer OR carinogens OR physiology OR biochemistry OR body weight OR anatomy OR body fluids OR metabolism OR immunology OR mutagens OR teratogenic compounds OR mutations OR antimetabolites) NOT (aquatic animals OR Invertebrates OR venomous animals OR wildlife OR biodegradation)) and ("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 Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR Hexolite) | Report Date: All dates	304 (7 selected for further consideration)
	2A3	distco:(dod) and key:((toxicity OR phramacokinetics OR toxicology OR pharmacology OR poisoning OR toxic hazards OR radiation hazards OR radiation effects OR toxic diseases OR toxic agents OR lethal agents OR antidotes OR death OR "signs and symptoms" OR cancer OR carinogens OR physiology OR biochemistry OR body weight OR anatomy OR body fluids OR metabolism OR immunology OR mutagens OR teratogenic compounds OR mutations OR antimetabolites) NOT (aquatic animals OR Invertebrates OR venomous animals OR wildlife OR biodegradation)) and ("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 Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR Hexolite) | Report Date: All dates	50 (3 selected for further consideration)

Database	Set #	Terms	Hits
DTIC R&E Gateway Search Date limit: 11/1/2012–1/23/2014	2B1	((toxicity OR phramacokinetics OR toxicology OR pharmacology OR poisoning OR toxic hazards OR radiation hazards OR radiation effects OR toxic diseases OR toxic agents OR lethal agents OR antidotes OR death OR "signs and symptoms" OR cancer OR carinogens OR physiology OR biochemistry OR body weight OR anatomy OR body fluids OR metabolism OR immunology OR mutagens OR teratogenic compounds OR mutations OR antimetabolites) NOT (aquatic animals OR Invertebrates OR venomous animals OR wildlife OR biodegradation)) and ("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 Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR Hexolite) Notes: Date limited to items created after October 31,2012. Searched all distribution limits individually: 6 results found in "approved for public release", 3 results found in "Gov't and Gov't contractors only"	9 (0 selected)

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

Selected Key Reference(s) or Sources	Date	Additional References Identified
Sweeney, LM; Gut, CP, Jr.; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling. Regul Toxicol Pharmacol 62(1):107−114. (forward search) 1 search result	3/2013	0 citations added
Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64(2):205−224 (backwards search) 0 search results	3/2013	0 citations added
Sweeney, LM; Gut, CP, Jr.; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling. Regul Toxicol Pharmacol 62(1):107−114. 35 cited papers	3/2013	0 citations added
Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64(2):205−224 69 cited papers	3/2013	3 citations added

Selected Key Reference(s) or Sources	Date	Additional References Identified
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) CalEPA (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) EPA – IRISTrack/New Assessments and Reviews (http://cfpub.epa.gov/ncea/iristrac/) to find dates (http://www.epa.gov/ncea/iris/index.html) to find data EPA NSCEP (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) IARC (http://monographs.iarc.fr/htdig/search.html) IPCS INCHEM (http://www.inchem.org/) NAS via NAP (http://www.nap.edu/) NCI (http://www.cancer.gov) NCTR (http://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/default.htm) National Institute for Environmental Health Sciences (NIEHS) http://www.niehs.nih.gov/ NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) NTP – RoC, status, results, and management reports (http://ntpsearch.niehs.nih.gov/query.html) WHO assessments – CICADS, EHC (http://www.who.int/ipcs/assessment/en/)	4/11/2012 1/27/2014	15 citations added 1 citation added

1

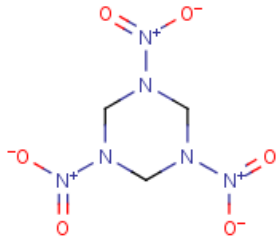
Table B-4. Summary disposition of DTIC database citations

Criteria	Percent of citations
Exclusion—Not chemical-specific	~50%
Exclusion—Bioremediation or biodegradation	5%
Exclusion—Chemical/physical properties of explosive properties	<5%
Exclusion—Physical or chemical treatment	<5%
Exclusion—Miscellaneous, including: • Superfund RODs for which the abstract did not specify whether RDX was a contaminant of concern • Meeting minutes and conference proceedings for which only general categories of topics were included in the DTIC record • DTIC records containing only a title containing inadequate information with which to classify the citation	~35%
Exclusion Total	*98% (847 total)*
Additional Resource—Regulatory documents	<5%
Additional Resource—Reviews	<5%
Additional Resource—Ecosystem effects	<5%
Additional Resource—Risk assessments	<5%
Additional Resource—Exposure levels	<5%
Additional Resource—Measurement methods	<5%
Additional Resource—Mixture only	<5%
Additional Resource—Toxicokinetics	<5%
Possible Further Review—No abstract	<5%
Possible Further Review—inadequate reporting in abstract	<5%
Inclusion Total	*~2% (20 total)*
TOTAL NUMBER OF DTICS CITATIONS (including 10 limited distribution for further review)	**867**

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

C.1. CHEMICAL PROPERTIES

Table C-1. Chemical identity and physicochemical properties of RDX

Characteristic or property	Value	Reference
Chemical structure		NLM, 2011
CASRN	121-82-4	
Color/form	White, crystalline solid	Bingham et al. (2001)
Molecular formula	$C_3H_6N_6O_6$	ACGIH (2001)
Molecular weight	222.12	Lide (2005)
Density (g/cm^3 at 20°C)	1.82	Lide (2005)
Melting point (°C)	205.5	Lide (2005)
Heat of formation (kJ/g)	−0.277	MG et al. (1984)
Log K_{OW}	0.87–0.90	Hansch et al. (1995)
K_{OC}	42–167	Spanggord et al. (1980)
Boiling point (°C)	276–280	Bingham et al. (2001)
Henry's law constant (atm-m^3/mole at 25°C)	2.0×10^{-1}	U.S. EPA (2003)
Solubility in water (mg/L at 25°C)	59.7	Yalkowsky and He (2003)
Vapor pressure (mm Hg at 20°C)	4.10×10^{-9}	Spanggord et al. (1980)

C.2. TOXICOKINETICS

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 transfered 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.2.1. Absorption

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

Oral Absorption

Quantitative information on blood levels following accidental exposure to RDX is limited to two studies of accidental oral exposures (Küçükardali et al., 2003; Woody et al., 1986) and one study of mixed dermal and inhalation exposure (Ozhan et al., 2003). A number of qualitative case studies of accidental exposures with similar toxic effects provide additional support that RDX is absorbed into the body (Hett and Fichtner, 2002; Harrell-Bruder and Hutchins, 1995; Goldberg et al., 1992; Ketel and Hughes, 1972; Hollander and Colbach, 1969; Stone et al., 1969). The oral absorption of RDX in humans was demonstrated in a case report of a 3-year-old male child who ingested plasticized RDX material that adhered to his mother's work boots and clothing (Woody et al., 1986). RDX was measured in serum, urine, cerebrospinal fluid, and feces. Based on a kinetic analysis of the serum RDX concentrations, the dose was estimated to be 85 mg/kg and the first-order absorption rate constants were estimated to be 0.34–2.20 $hour^{-1}$ (Woody et al., 1986)[2]. Sweeney et al. (2012a) estimated the absorption rate constant for this same subject to be 0.060 $hour^{-1}$. The large range in the calculated absorption rate constants resulted from uncertainty in the dose and time to peak serum RDX levels, and the models that were used to simulate the RDX toxicokinetics. Ozhan et al. (2003) summarized plasma RDX levels in five military personnel who

[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 $hour^{-1}$.

were accidentally exposed to toxic levels of RDX. Although Ozhan et al. (2003) reported that personnel were exposed through dermal and inhaled routes, secondary oral exposure may have occurred. Based on PBPK model fits to the plasma RDX concentration data, Sweeney et al. (2012a) estimated a first-order absorption rate constant of 0.033 $hour^{-1}$. Küçükardali et al. (2003) summarized plasma RDX levels in five military personnel who ingested toxic levels of RDX (doses were not reported). RDX was detected in plasma of all patients within 3 hours after ingestion.

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

An estimate of oral bioavailability in rats can also be obtained from data on blood RDX concentrations reported in Krishnan et al. (2009). Male Sprague-Dawley rats received a single intravenous (i.v.) (0.77 or 1.04 mg/kg) or oral (1.53 or 2.07 mg/kg, dissolved in water) dose of RDX. Estimates of bioavailability were obtained based on the reported blood RDX concentrations, terminal elimination rate constants (estimated for this review by fitting the serum RDX data with a first-order exponential function, see Table C-4 in the Elimination section below) and the blood area under the curve (AUC) values (calculated for this review using the trapezoid rule extrapolated to infinite time). Calculated dose-adjusted AUC values were 9.6 and 8.4 hours-kg/L for the i.v. studies and 4.7 and 6.0 hours-kg/L for the oral dosing studies. These AUC values correspond to estimated oral bioavailability ranging from 50 to 70%. Recovery of administered radiolabel was incomplete (~90% of the administered carbon-14) in the studies (Schneider et al., 1978, 1977); therefore, it is possible that oral bioavailability is actually higher than 83–87%. Guo et al. (1985) reported data on blood tritium kinetics in mice that received i.v. (0.055 mg RDX or ~2.5 mg/kg body weight) or oral doses (50 mg/kg) of [^{3}H]-RDX. Based on the reported blood tritium concentrations (% of dose/g) and terminal $t_{1/2}$ values for concentrations of tritium in blood (1.1 days for i.v. and 2.2 days for oral), the corresponding AUCs of the blood concentration versus time curves were calculated (calculated for this review using the trapezoid rule extrapolated to infinite time) to be 30 and

16 hours-% dose/g for i.v. and oral dosing, respectively. This corresponds to an oral bioavailability of RDX-derived tritium concentration of approximately 50% (i.e., 16/30).

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

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

Oral bioavailability of RDX is lower when administered as RDX-contaminated soil or when RDX is in plant materials that were grown on RDX-contaminated soils. Crouse et al. (2008) investigated the oral bioavailability of RDX in contaminated soils relative to pure RDX by comparison of the AUC for the RDX blood concentration versus time curves. Adult male Sprague-Dawley rats were administered oral doses (in gelatin capsules) of pure RDX (99.9% purity; neat) or an equivalent amount of RDX in contaminated soils from the Louisiana Army Ammunition Plant (LAAP) or Fort Meade (FM). Blood concentrations for rats dosed with LAAP 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 LAAP soil than for neat RDX ($p \leq 0.003$ for AUC), suggesting that oral bioavailability of RDX from LAAP soil was 25% lower than neat RDX. For FM 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 FM soil was approximately 15% lower than for neat RDX, although the AUC for FM 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 (50mg/kg in methyl cellulose) (Guo et al., 1985). The tritium radiolabel was detected in blood 15 minutes following dosing and the highest concentrations in blood were observed 30 minutes after dosing. Based on an analysis of the blood tritium concentration kinetics, the authors estimated an absorption rate constant of 8.7 hour^{-1}. In Sprague-Dawley rats administered single doses (0.2–18.0 mg/kg) of RDX in gelatin capsules, peak blood RDX concentrations were observed between 2.5 and 6 hours (Bannon et al., 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 swine administered single doses (5–10 mg/kg) of finally powdered (>98% pure) RDX in gelatin capsules occurred at 3–8 hours after dosing (Bannon et al., 2009), compared to 24 hours after a single dose (100 mg/kg) of RDX administered as a finely powdered in saline (Bannon et al., 2009; Schneider et al., 1977). Peak plasma concentrations in Yucatan miniature swine administered a single dose of [^{14}C]-RDX (45 mg/kg as a suspension in carboxymethylcellulose) were reached within 6–12 hours after dosing (Musick et al., 2010). Krishnan et al. (2009) and Sweeney et al. (2012a) estimated absorption rates in rats dosed with higher doses of coarse granular RDX to be approximately 100 times slower than absorption rates in rats dosed with lower doses of finely powdered neat RDX preparations or neat RDX dissolved in aqueous vehicles. For example, Krishnan et al. (2009) estimated the absorption rate constant to be 0.75 hour^{-1} for rats dosed with neat RDX dissolved in water (1.53 or 2.07 mg/kg) or neat RDX in a gelatin capsule (0.2 or

1.24 mg/kg, Crouse et al. (2008)), compared to 0.0075 hour^{-1} for rats dosed with coarse granular RDX (100 mg/kg, Schneider et al. (1977)).

Inhalation Absorption

Studies evaluating absorption of RDX in humans following inhalation exposure were not identified. Several case reports have documented seizures and other neurological effects in individuals exposed to RDX either in a manufacturing setting or in the course of using RDX as a cooking fuel (Testud et al., 1996b; Testud et al., 1996a; 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.2.2. 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; Ozhan et al., 2003; Woody et al., 1986). Concentrations of RDX in serum and cerebrospinal fluid were similar (11 and 9 mg/L, respectively) in a child 24 hours after ingesting an estimated dose of 85 mg/kg RDX (Woody et al., 1986). More extensive information on tissue distribution is available for animals, including mice, rats, and swine (Musick et al., 2010; Bannon, 2006; Reddy et al., 1989; Guo et al., 1985; MacPhail et al., 1985; Schneider et al., 1977). In

these studies, RDX or radiolabeled RDX ([^{14}C] or [^{3}H) was administered by the oral, intraperitoneal (i.p.), i.v., or intratracheal route and the distribution of the RDX or radiolabel was measured. Since metabolism of RDX can result in loss of carbon-14 or tritium from the parent compound, the distribution of radiolabel will not necessarily reflect the distribution of RDX (Schneider et al., 1977). To compare tissue distributions in studies in which animals received different doses by different routes of administration, distribution data are expressed as ratios of tissue RDX or radiolabel to that of either whole blood or plasma, whichever was reported. RDX in blood distributes into red blood cells 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 plasma:red blood cell RDX ratios of approximately 1.0 (Krishnan et al., 2009). As a result of the similarity between plasma and whole blood concentrations, tissue distribution is approximately equivalent when expressed as ratios of blood or plasma.

Studies conducted in rats, mice, and swine indicate that absorbed RDX distributes to many different tissues. Schneider et al. (1977) estimated the volume of distribution of RDX to be approximately 2.18 L/kg in rats, based on plasma RDX kinetics in rats that received a single i.p. dose of RDX (5–6 mg/kg). Consistent with this estimate are observations of tissue:blood (or plasma) concentration ratios that exceed 1 in various tissues, including brain (showing that RDX can cross the blood:brain barrier), heart, kidney, and liver (Musick et al., 2010; Bannon et al., 2006; MacPhail et al., 1985; Schneider et al., 1977). Distribution within the brain may not be uniform. However, Bannon et al. (2006) observed tissue:blood concentrations for RDX of approximately 4 in brain hippocampus and 3.5 in brain cortex of swine that received a single oral dose of 10 mg/kg [^{14}C]-RDX, although this is the only study that reported distribution for brain regions. Reported tissue:blood (or plasma) concentration ratios of RDX 24 hours following a single dose (oral or i.p.) were 1–9 for kidney, 1–7 for liver, and 1–3 for heart (Table C-2; Bannon (2006); (Schneider et al., 1977). With repeated oral dosing (e.g., 30–90 days), tissue:blood ratios of RDX for these tissues are consistently greater than unity (Schneider et al., 1978). There is no consistent evidence that RDX accumulates in fat, although estimates of the fat:blood partition coefficient range from 6 to 8 and exceed that of other tissues (Sweeney et al., 2012a; Krishnan et al., 2009).

Table C-2. Distribution of RDX or radiolabel from administered RDX[a]

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

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

In rats, RDX can cross the placental:blood barrier resulting in exposure to the fetus, and can also be transported into maternal milk. Hess-Ruth et al. (2007) detected RDX in the brain tissue of postnatal day (PND) 1 rat pups (concentrations ranged from 0.64 to 7.6 µg/g brain tissue, with no differences between males and females) after maternal exposure to 6 mg/kg RDX via gavage from gestation day 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.2.3. Metabolism

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

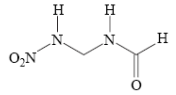
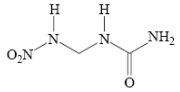
RDX undergoes metabolism through processes that generate CO_2. In Sprague-Dawley rats administered a single 50 mg/kg gavage dose of [^{14}C]-RDX, 43% was recovered as exhaled [$^{14}CO_2$] after four days (Schneider et al., 1977). Similarly, approximately 30–50% of the radioactivity was recovered as exhaled [$^{14}CO_2$] in rats administered [^{14}C]-RDX in saturated drinking water or daily gavage for up to three months (Schneider et al., 1978). Metabolism of RDX to CO_2 was also observed in prairie voles following dietary exposure (average RDX dose per animal of 2.3 mg/kg-day) to [^{14}C]-RDX incorporated plant materials for 5–7 days, with approximately 9% of the administered [^{14}C]-RDX dose eliminated as exhaled [$^{14}CO_2$] (Fellows et al., 2006).

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

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

and mortality) as determined through identification of the median lethal dose using the EPA up-and-down procedure in deer mice of varying ages (Smith et al., 2009; Rispin et al., 2002).

Table C-3. Principal urinary metabolites of RDX in miniature swine 24 hours after dosing with RDX

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

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

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

C.2.4. Excretion

The primary routes of elimination of absorbed RDX are excretion of RDX and metabolites in urine, and exhalation of CO_2 liberated from metabolism of RDX (Sweeney et al., 2012a; Musick et al., 2010; Krishnan et al., 2009; Major et al., 2007; Schneider et al., 1977). Tritium derived from administered [^{3}H]-RDX has been detected in mouse gall bladder contents, suggesting biliary secretion in this species (Guo et al., 1985); however, biliary secretion of RDX or metabolites has not been confirmed in other animal species. Studies conducted in the rat and swine suggest that metabolism is the dominant mechanism of elimination of absorbed RDX. In both species, metabolites dominated the carbon-14 distribution in urine of animals that received doses of [^{14}C]-RDX, with RDX accounting for <5% of the urinary carbon-14 (Musick et al., 2010; Schneider et al., 1977).

Data on kinetics of elimination of absorbed RDX from blood are available from reports of accidental exposures of humans to RDX (Table C-4). Woody et al. (1986) estimated the elimination

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

Table C-4. Elimination $t_{1/2}$ values for RDX or radiolabeled RDX

Animal	Route	Dose (mg/kg)	Time[a]	$t_{1/2}$ (hrs)	Source
Human (child)	Oral	85[b]	24–120 hrs	15.0[c]	Woody et al. (1986)
Human (adult)	Oral	NA	24–96 hrs	21–29[c,d]	Ozhan 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	i.v.	2.5	1.5 min–24 hrs	26[d,e]	Guo et al. (1985)
Mouse	Oral	50	1.5 min–168 hrs	53[d.e]	Guo et al. (1985)

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

The kinetics of elimination of absorbed RDX from blood has been evaluated in rats and mice; in both species, elimination kinetics were bi-phasic (Krishnan et al., 2009; Guo et al., 1985; Schneider et al., 1977). As shown in Table C-4, estimated $t_{1/2}$ values for the terminal elimination phase in rats range from 5 to 10 hours (Krishnan et al., 2009; Schneider et al., 1977). Guo et al. (1985) estimated the terminal elimination $t_{1/2}$ for RDX-derived tritium in mice to be approximately 26 hours following a single i.v. dose of [^{3}H]-RDX (2.5 mg/kg). The elimination $t_{1/2}$ for tritium in mice following an oral dose of [^{3}H]-RDX (50 mg/kg) was approximately 53 hours (Guo et al., 1985). The shorter elimination $t_{1/2}$ estimated for rats (Krishnan et al., 2009; Schneider et al., 1977) compared to mice (Guo et al., 1985) may reflect a real species difference in elimination kinetics of RDX, or may reflect a difference between the kinetics of elimination of RDX and of tritium derived from [^{3}H]-RDX, which would include RDX metabolites.

C.2.5. Physiologically-Based Pharmacokinetic (PBPK) Models

Overview of Available PBPK Models

A PBPK model to simulate the pharmacokinetics of RDX in rats was first developed by Krishnan et al. (2009) and improved upon to extend the model to humans and mice (Sweeney et al., 2012a; Sweeney et al., 2012b). The Sweeney et al. (2012a) model consists of six main compartments: blood, brain, fat, liver, and lumped compartments for rapidly perfused tissues and slowly perfused tissues (Figure C-1). The model can simulate RDX exposures via the IV 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 gastrointestinal tract into the liver, with 100% of the dose absorbed. RDX is assumed to be cleared by first-order metabolism in the liver. However, there is no representation of the kinetics of any RDX metabolites. The acslX model code (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, Alabama) was obtained from the authors of Sweeney et al. (2012a).

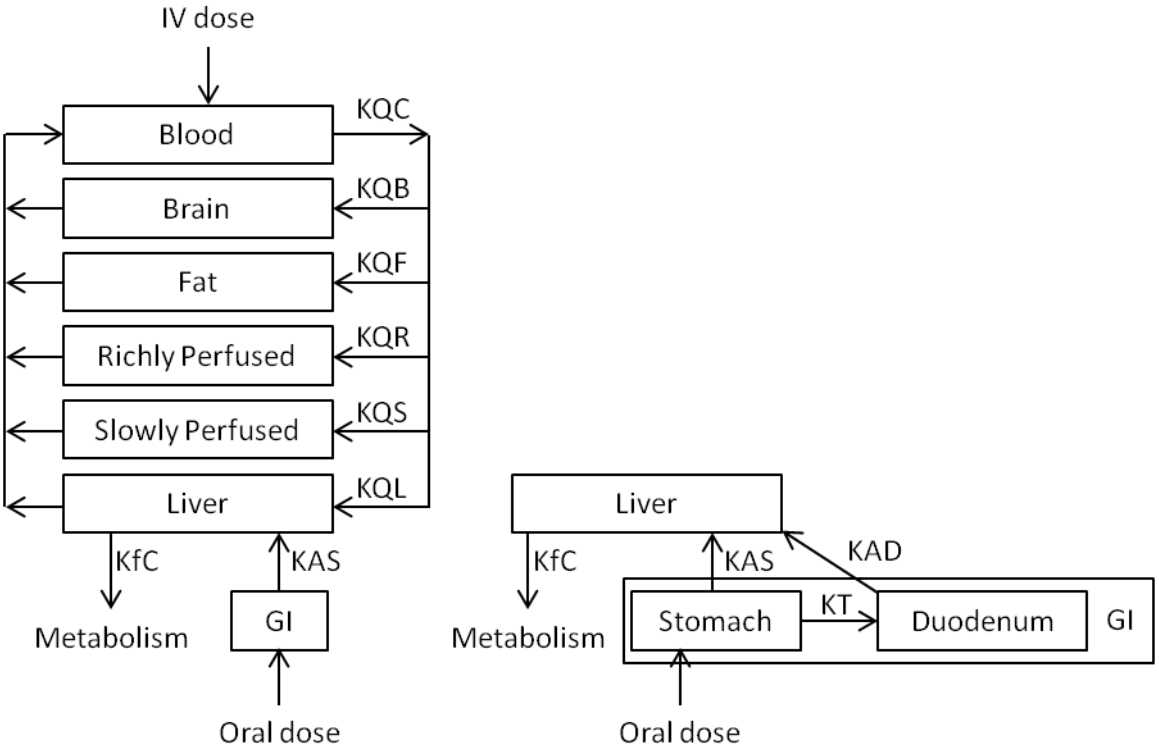


Figure C-1. PBPK model structure for RDX in rats and humans. Exposure to RDX is by the IV or oral route and clearance occurs by metabolism in the liver. See Table C-5 for definitions of parameter abbreviations. The GI tract is represented as 1-compartment in Krishnan et al. (2009) (on the left) and 2-compartments 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-5. 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 IV 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) of 1.0, the optimized values for PF and KfC were fairly similar to those used by Krishnan et al. (2009) of 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 IV exposure (Schneider et al., 1977).

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 1-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 2-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 1-compartment model. The value of KAS was adjusted separately to fit the oral studies with 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 (Ozhan et al., 2003). The amounts of RDX ingested in both studies were unknown, so Sweeney et al. (2012a) estimated the dose amount by optimizing this parameter to fit the data (Table C-5). 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 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, 2008; Lipscomb and Poet, 2008). The scaling from rats yielded a human *in vivo* metabolic rate constant of 12.4 kg-bw$^{0.33}$/hour which is similar to the values Sweeney et al. (2012a) derived by fitting the combined Ozhan 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 rate constant for metabolism (KfC), and the oral absorption rate constant (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 kg0$^{.33}$/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.

Table C-5. Parameter values used in the Sweeney et al. (2012a) and Sweeney et al. (2012b) PBPK models for RDX in rats, humans, and mice

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Body weight (BW; kg)	0.3	70	0.0206	Default values; study-specific values used if available
Cardiac output (KQC, L/h/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)

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
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 PC (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 (adults)	102	Sweeney et al. (2012a)[b,c]; Sweeney et al. (2012b)[d]
GI absorption				
Dosing via gavage				
Absorption from compartment 1 (KAS, /hr)	0.83	0.033	0.51	Sweeney et al. (2012a)[c,d,e]
Transfer from compartment 1 to compartment 2 (KT, /hr)	1.37	0	0	Sweeney et al. (2012a)[c,d]
Absorption from compartment 2 (KAD, /hr)	0.0258	0	0	Sweeney et al. (2012a)[c,d]
Dosing via capsule (KAS, /hr)	0.12	NA	NA	Sweeney et al. (2012a)[e]
"coarse" RDX formulation (KAS, /hr)	0.005	NA	NA	Sweeney et al. (2012a)[e]

[a]Predicted from n-octanol:water PC.
[b]Optimized from rat IV data.
[c]Optimized from human data of Ozhan 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 above PBPK model was evaluated and subsequently modified by EPA for use in dose-response modeling in this assessment. This is detailed in the following section.

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

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

1) The model code and the parameter values matched the published reports.

2) The absorption of RDX from the GI tract did not use a consistent structure; for gavage doses the model used a 2-compartment GI submodel and for other oral exposures (e.g., gelatin capsule) the model used a 1-compartment GI submodel. The model was revised to have a 1-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 independent comparison against model predictions.

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

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

The Sweeney et al. (2012a) model for rats was modified by changing the oral absorption rate constants (as discussed below) and the partition coefficients for the fat and slowly perfused tissues (PF and PS) as shown in Table C-6. 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 iv exposure (Figure C-2) are slightly worse than the Sweeney et al. (2012a) rat model because the Sweeney et al. (2012a) rat model optimized the fat:blood and slowly perfused tissue partition coefficients to fit the data.

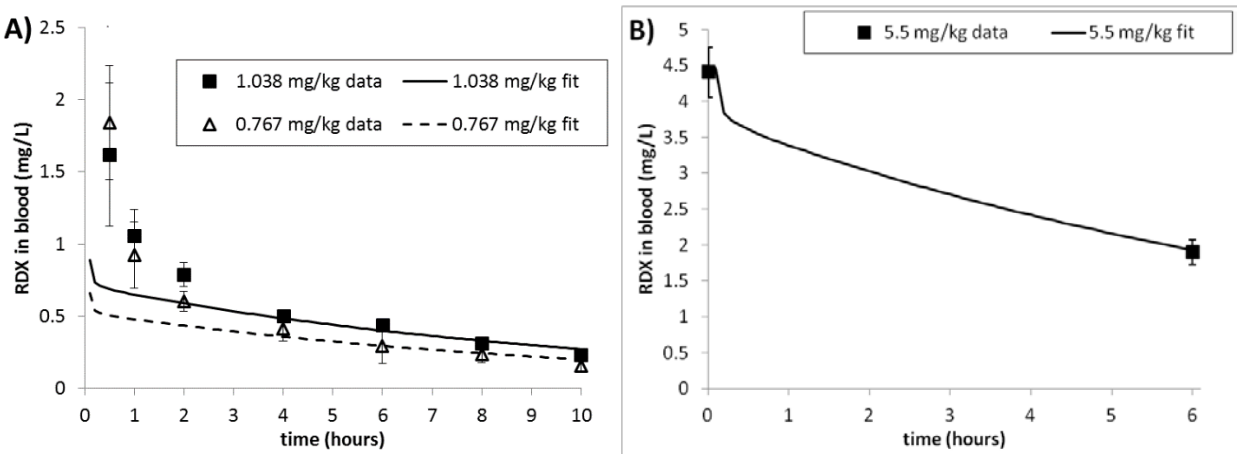


Figure C-2. EPA rat PBPK model predictions fitted to observed RDX blood concentrations in male and female SD rats following intravenous exposure. A) data from Krishnan et al. (2009) (0.4 kg rats) and B) data from Schneider et al. (1977) (simulation of 0.25 kg rats and 5.5 mg/kg dose for 0.2–0.25 kg rats and 5–6 mg/kg dose).

Table C-6. Parameters values used in the EPA application of the rat, human, and mouse models

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
Body weight (BW; kg)	0.3	70	0.0206	Default values shown; study-specific values used if available
Cardiac output (KQC; L/h/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	0.052	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)				

Parameter (abbreviation; units)	Rat	Human	Mouse	Source
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 and metabolism				
Liver (PL)	1.2	1.3	1.3	Krishnan et al. (2009)[a]
Brain (PB)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Richly perfused tissues (PR)	1.4	1.6	1.6	Krishnan et al. (2009)[a]
Fat:blood PC (PF)	7.55	7.55	7.55	Krishnan et al. (2009)[a]
Slowly perfused tissues (PS)	1.0	1.0	0.9	Krishnan et al. (2009)a
First-order metabolic rate constant (KfC; $kg^{0.33}$/hr)	2.6	9.87(small boy); 11.2(soldiers)	77	Sweeney et al. (2012a)[b,c]; Sweeney et al. (2012b)[d]
Absorption				
Absorption from GI to liver (KAS; /hr)	Table C-7	1.75	0.6	Fit to rat, human, and mouse oral data
Absorption from lung to blood (Klung; /hr)		0.75		Fit to human data

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

Absorption of RDX from the GI Tract

As discussed above in the oral absorption section under toxicokinetics (Section C.2.1) 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 coarse granular RDX and 50 mg/kg finely powdered RDX and observing 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 1-compartment GI tract sub-model 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 and the model fits obtained with the EPA's revised parameters for rats are shown in Figure C-3 to C-5. The oral dosing formulations were grouped into four categories; RDX dissolved in water, RDX in capsules, fine grain and coarse grain RDX. The absorption rate constant for RDX dissolved in water was optimized to the data in the Krishnan et al. (2009) study (Figure C-3). The absorption rate constant for RDX in capsules was optimized to the data in the Crouse et al. (2008) and Bannon et al. (2009) studies (Figure C-4). The absorption rate constant for fine grain RDX was optimized to the data described below (*Additional RDX time-course data)* in the MacPhail et al. (1985) and Schneider et al. (1977) studies (Figure C-7). The Schneider et al. (1977) study was used to estimate the absorption rate constants for coarse grain RDX (Figure C-5; as represented by the fit to the data obtained by the solid curve at 100% bioavailability). Overall, the fits of the EPA revised model to the blood time-course data of these studies are similar to the fits of theSweeney et al. (2012a) rat model. The fits to RDX brain time course data after oral exposure to RDX in capsules (Figure C-6A) are similar to the fits of the Sweeney et al. (2012a) rat model. The absorption rate constants for each dosing formulation are listed in Table C-7.

An alternative to varying the absorption rate constant (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, S*ensitivity analysis of the rat PBPK model*.

Table C-7. 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.

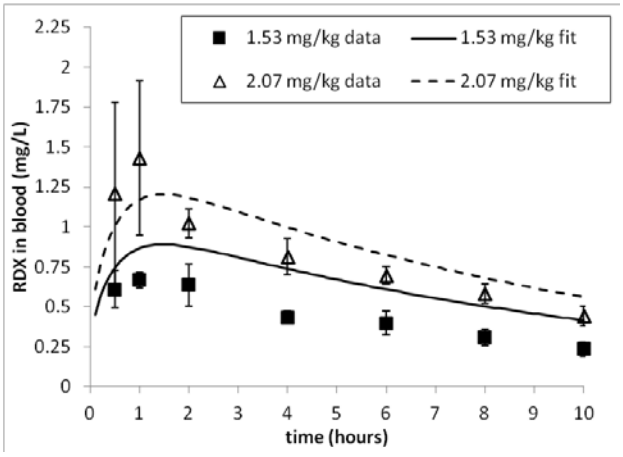


Figure C-3. EPA rat PBPK model predictions fitted to observed RDX blood concentrations following oral exposure to RDX dissolved in water. Male and female SD rats (0.4 kg) were dosed by gavage (Krishnan et al., 2009).

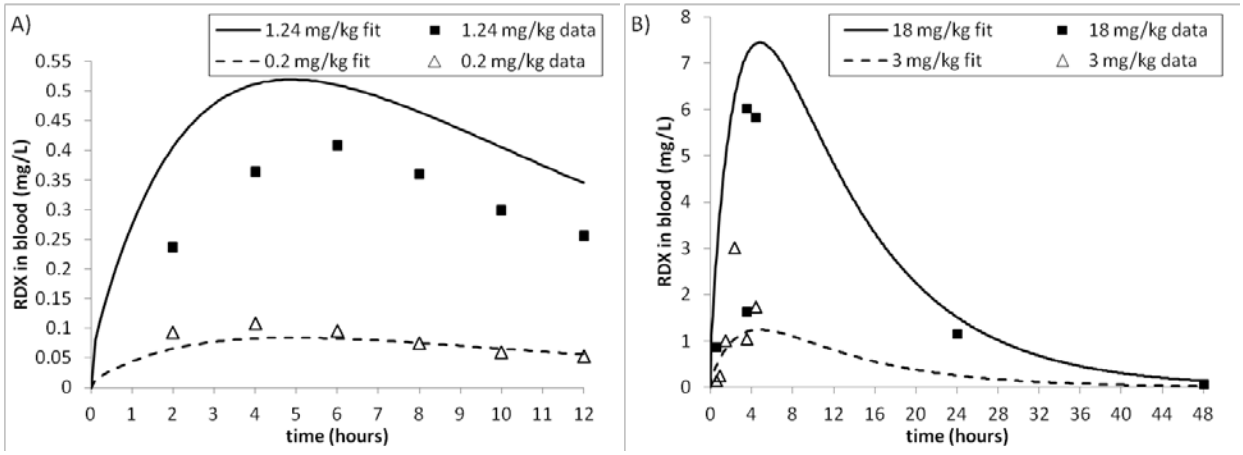


Figure C-4. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to RDX in dry capsules. The ingested RDX doses were A) 0.2 and 1.24 mg/kg RDX in male SD rats (0.4 kg, data from Crouse et al. (2008)) and B) 3 and 18 mg/kg RDX in male and female SD rats (0.35 kg, data from Bannon et al. (2009)) for KAS = 0.35/hour.

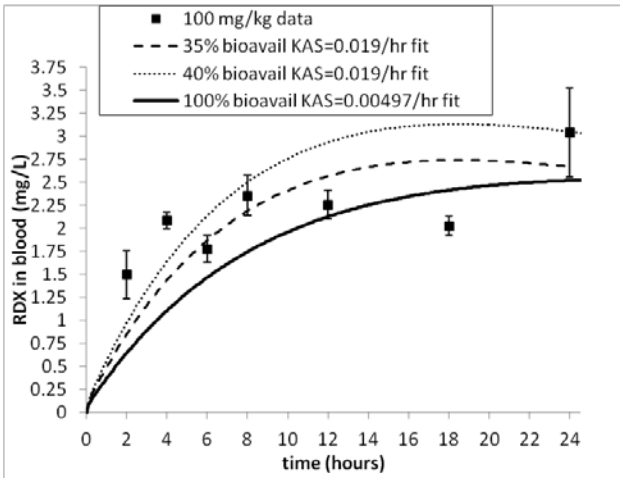


Figure C-5. Effect of varying oral absorption parameters on EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to coarse grain RDX. Symbols denote observed RDX blood concentrations measured in male SD rats (0.225 kg) resulting from oral doses of 100 mg/kg RDX (Schneider et al., 1977). The absorption rate constant (KAS) fit to these data assuming 100% bioavailability resulted in the same estimate (KAS = 0.00497/hour) as obtained by Sweeney et al. (2012a). Alternatively, for KAS fixed at the value fit to fine grain RDX in a saline slurry (KAS = 0.019/hour fit to data from Schneider et al. (1977) and MacPhail et al. (1985), Figure C-7) the estimate of oral bioavailability fit to the RDX blood concentrations was 35%. A bioavailability of 40% and KAS = 0.019/hour is also shown for comparison.

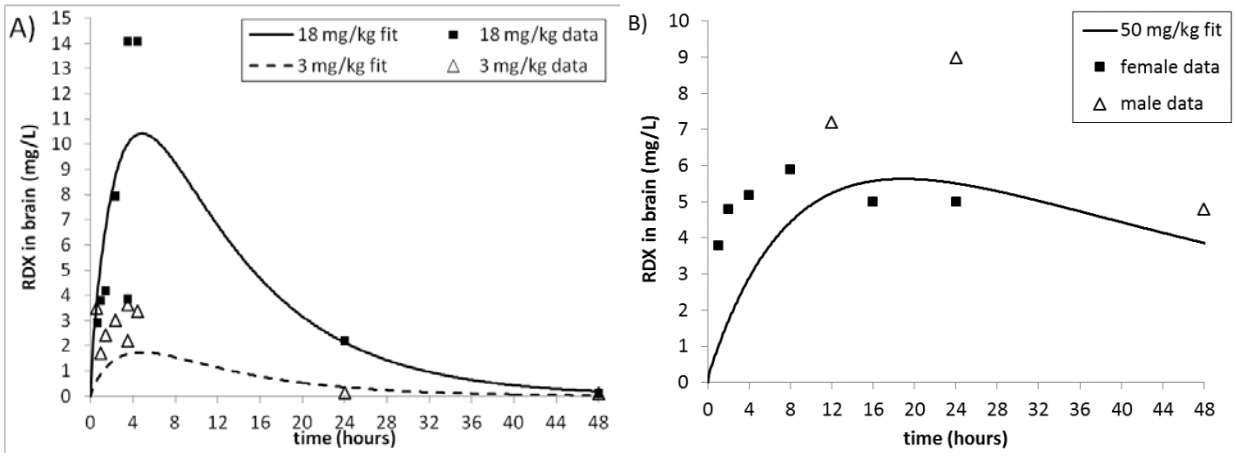


Figure C-6. EPA rat model predictions fitted to observed RDX brain tissue concentrations following oral exposure to RDX. A) 3 and 18 mg/kg RDX in dry capsules (0.35 kg male and female rats data from Bannon et al. (2009)); best fit KAS = 0.35/hour. B) 50 mg/kg fine grain RDX in a saline slurry (0.25 kg male and female rats data from MacPhail et al. (1985)); best fit KAS = 0.019/hour.

Additional RDX Time-Course Data

The EPA revised models were simultaneously fitted against additional RDX time course data (not used in the original Sweeney et al. (2012a) model calibration) from two studies in which animals received oral doses of fine grain RDX (MacPhail et al., 1985; Schneider et al., 1977) in Figure C-7 and RDX brain time course data from a study in which animals received oral doses of fine grain RDX (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 therefore likely provides a more robust estimated parameter.

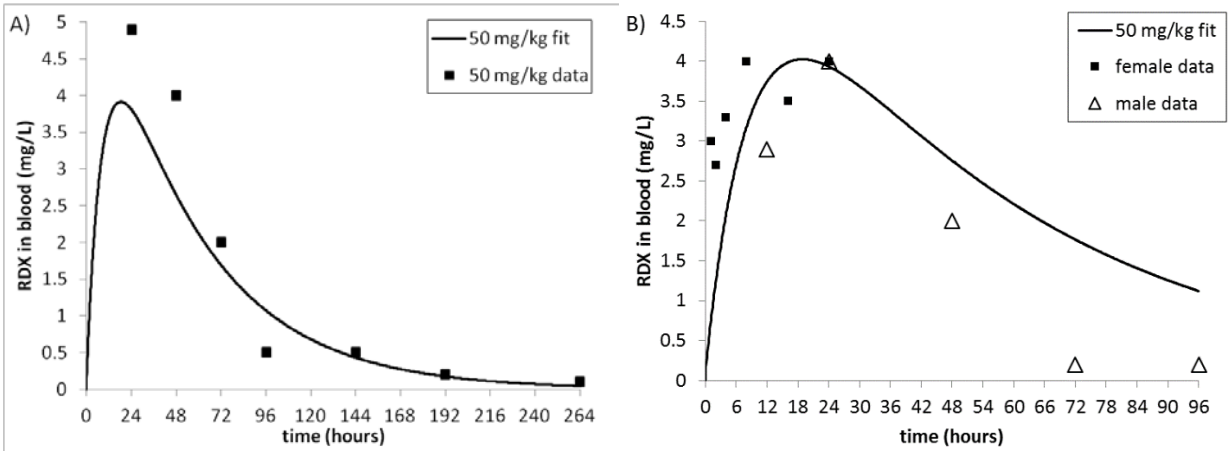


Figure C-7. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to fine grain RDX in a saline slurry. Oral doses of 50 mg/kg RDX were administered to A) male SD rats (0.225 kg) (Schneider et al., 1977) and B) male and female SD rats (0.25 kg) data (MacPhail et 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 where 20 mg/kg RDX was administered in saline slurry and a drinking water study where rats were provided with RDX-saturated drinking water (50–70 µg/mL) *ad libitum* for which the study authors estimated a daily dose between 5 and 8 mg RDX/kg BW. It is striking that the observed RDX blood concentrations in the gavage study (Figure C-8, symbols) were virtually the same as or only slightly elevated compared to the blood concentrations reported in the drinking water exposures, with an approximately threefold lower daily administered dose in the drinking water study (Figure C-9, symbols). This is counter to the expectation that higher doses cause higher blood levels and is discussed further below.

EPA's modified PBPK model was set up to simulate drinking water exposures with a non-continuous sipping pattern based on Spiteri (1982) which assumed 80% of the consumption to

occur episodically at night when the rats were awake[3]. The model predicts blood concentrations to increase in proportion to the total dose; for the gavage study the model predictions yielded an RDX blood concentration approximately threefold higher than the reported mean blood concentrations (Figure C-8) while for the subchronic drinking water study the model fit the data reasonably well (Figure C-9).

It is possible that multiple mechanisms such as elimination of unabsorbed RDX or metabolic induction may explain why the observed RDX blood concentrations did not increase in proportion to the higher administered dose in the gavage studies compared to the drinking water. 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 micrograms RDX per gram 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 RDX blood concentrations seen. The reasonably good fits to the model to the drinking water data set demonstrated achievement of regular periodic levels, 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 mg/kg-day vs. 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 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.

[3]A constant drinking water ingestion rate interspaced between episodes of no ingestion was assumed. Each 12-hour awake period consisted of 8 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 4 cycles with regular sipping periods of 30 minutes followed by 2.5 hours of no ingestion.

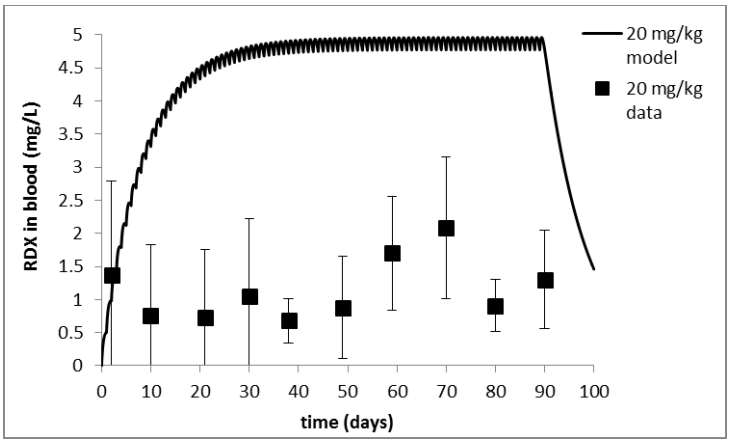


Figure C-8. Comparison of EPA rat model predictions with data from Schneider et al. (1978) **for the subchronic gavage study.** Model fits and mean observed RDX blood concentrations resulting from daily gavage doses of 20 mg/kg RDX for 90 days to male and female SD rats (0.225 kg). The RDX in saline slurry was assumed to be coarse grained with oral absorption rate constant KAS = 0.00497/hour.

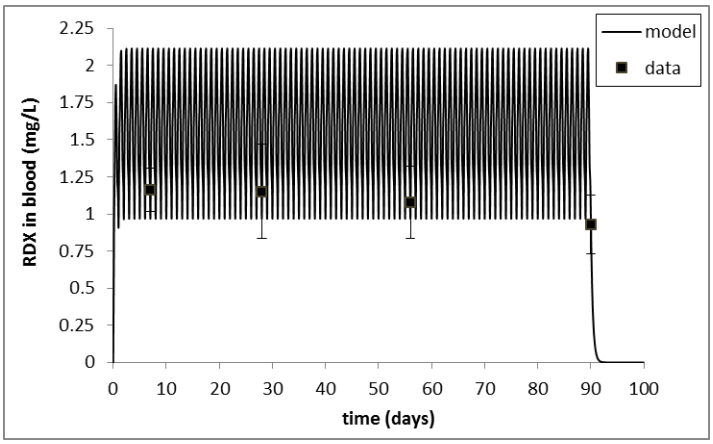


Figure C-9. Comparison of EPA rat model predictions with data from Schneider et al. (1978) **for the subchronic drinking water study.** Model fits and mean observed RDX blood concentrations resulting from a daily estimated dose of 6.5 mg RDX/kg-day for 90 days to male and female SD rats (0.225 kg). The 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).

Simulating Exposures in Humans

The Sweeney et al. (2012a) model for humans was modified in the same ways as the rats, by changing the partition coefficients for the fat and slowly perfused tissues (PF and PS) as shown in Table C-6 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 Ozhan et al. (2003) the RDX doses were unknown and so the doses were 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 Ozhan et al. (2003) data using the best fit values of an oral dose = 3.5 mg/kg, KAS = 1.75/hour, and KfC = 9.87 $kg^{0.33}$/hour are shown in Figure C-11.

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 Ozhan 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 therefore was not modeled. This decision is supported by a study that used excised human skin and reported 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 Ozhan 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 100% of the inhaled dose was absorbed and 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 Ozhan et al. (2003) as shown in Figure C-11.

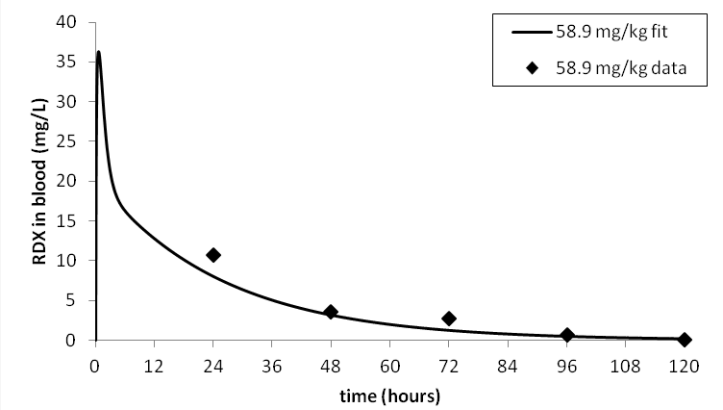


Figure C-10. EPA human model predictions fitted to observed RDX blood concentrations resulting from an accidental ingestion of RDX by a 14.5-kg boy (Woody et al., 1986). The best fit values were KAS = 1.75/hour, dose = 58.9 mg/kg and KfC = 9.87 $kg^{0.33}$/hour.

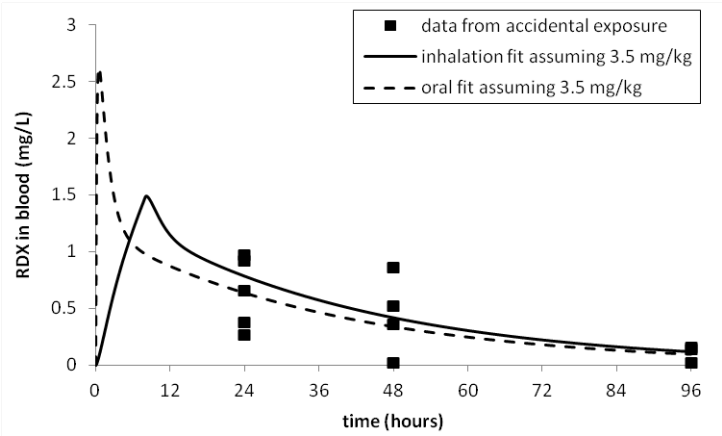


Figure C-11. EPA human model predictions fitted to observed RDX blood concentrations resulting from accidental exposure to adults assumed to be 70 kg (Ozhan et al., 2003). For an assumed oral exposure the best fit values were KAS = 1.75/hour, dose = 3.5 mg/kg and KfC = 9.87 $kg^{0.33}$/hour. For the same 3.5 mg/kg dose and metabolism rate constant an inhalation exposure found a best fit value for Klung of 0.75/hour.

Sensitivity Analysis of the Rat and Human PBPK Models

A sensitivity analysis was performed to see how each model parameter affects the model output. A sensitivity coefficient, defined as the change in a specified dose metric due to a 1% increase in the value of a parameter, was calculated for each parameter in the rat and human models. This analysis was carried out for both short (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 area-under-the-curve (AUC). Parameters with sensitivity coefficients

greater than 0.1 in absolute value (i.e., considered sensitive) are presented in Table C-8. For the blood AUC dose metric the only sensitive RDX-specific parameter is the metabolic clearance rate (KfC). This sensitivity is likely because bioavailability was assumed to be 100% and metabolism is the only route of elimination in the model. These assumptions mean that all administered RDX will be absorbed and metabolized, in other words the blood AUC is proportional to the dose and inversely proportional to the metabolic clearance rate constant. For the parameter values in this model the rate of metabolism is relatively slow compared to the transport of RDX between other tissues and the site of metabolism in the liver, so that the blood AUC is not sensitive to parameters that impact transport such as KQC and KQL. Because the metabolic clearance rate constant is scaled to BW and by liver volume, the blood AUC is also sensitive to these parameters. The sensitivity analysis by Sweeney et al. (2012b) for the AUC of RDX in the liver found the model was sensitive to the liver:blood partition coefficient (PL) in addition to the same parameters (KfC, KVL and BW) found for the blood AUC.

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 (C.2.1), estimates of the bioavailability of RDX range from 50 to 87% or greater and may depend upon the physical form of RDX (Krishnan et al., 2009; Schneider et al., 1978, 1977). However, as seen in Figure C-5, it was not possible to identify the bioavailability and the absorption rate (KAS) as separate parameters by fitting to the available RDX blood concentration time course. Introducing oral bioavailability as an additional unknown parameter and recalibrating the model did not appear to provide an advantage. Therefore, 100% bioavailability was assumed in the model and acknowledged as an uncertainty.

Table C-8. Sensitivity coefficients for rat and human RDX PBPK models

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

Parameters with sensitivity coefficients < 0.1 in absolute value are considered not sensitive, and are listed below:
- cardiac output (KQC);
- fractional blood flow to all tissues (liver, KQL; fat KQF; slowly perfused tissues KQS; brain KQB);
- fractional tissue volume of fat (KVF), brain (KVB), and blood volume (KVV);
- blood partition coefficients to all tissues (liver PL, fat PF, rapidly perfused PR, slowly perfused PS, brain PB);
- absorption rates from GI (KAS, KT, KAD)

Simulating Exposures in Mice

Physiological parameters specific to mice were obtained from the literature (Brown et al., 1997) and are shown in Table C-6. The partition coefficients calculated for mice by Sweeney et al.

(2012b) were used, which includes 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 iv 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.

The only 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, 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.

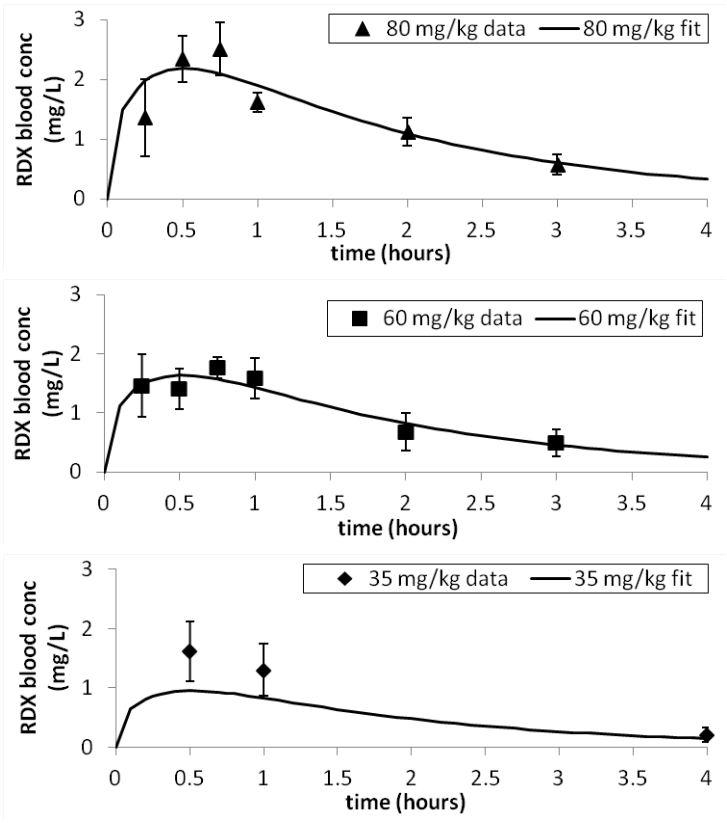


Figure C-12**. Comparison of EPA mouse PBPK model predictions with data from oral exposure to RDX dissolved in water.** Model fits and mean and standard deviation of observed RDX blood concentrations in female B6C3F$_1$ mice

(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).

Summary of Confidence in PBPK Models for RDX

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

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

There are several uncertainties in these models, listed below, the most significant of which pertain to the mouse PBPK model. The mouse model was based on a single data set, which used high RDX doses to obtain detectable RDX blood concentrations, and the type 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 the lack of in vitro measurements of RDX metabolism by mouse cells and lack of quantification of potential routes of RDX elimination in mice. Overall these uncertainties result in lower confidence in the mouse model than in the rat an 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 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 at which RDX was detected was 35 mg/kg; this concentration was high enough to manifest some toxicity in the chronic mouse bioassay. The measured blood concentration at the 35 mg/kg dose is based on the level measured from one single animal (others were non-detects or excluded as outliers). This 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 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 CYP P450 enzymes. Alternatively, mice may have other unknown metabolic pathways responsible for metabolizing RDX. Identifying the specific CYP enzymes, 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 confidence in using the mouse PBPK model for predicting mouse blood RDX concentrations.

Model Code for RDX PBPK Model Used in the Assessment

The PBPK acslX model code is made available electronically through the HERO database. All model files may be downloaded in a zipped workspace from HERO at https://hero.epa.gov/index.cfm/project/page/project_id/2216; search for "RDX PBPK files in acsIX format."

C.3. HUMAN STUDIES

Table C-9 presents a summary of case reports of humans acutely exposed to RDX. Table C-10 provides a summary of the methodologic features of the available epidemiology studies of RDX.

1 **Table C-9. Summary of case reports of exposure to RDX**

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Barsotti and Crotti (1949) 17 males among 20 male Italian workers (1939–1942) Manufacturing	Inhalation of RDX powder during the drying, cooling, sieving, and packing processes of its manufacture	Generalized convulsions of a tonic-clonic (epileptic) type followed by postictal coma; loss of consciousness without convulsions; vertigo; vomiting and confusion; transient arterial hypertension Symptoms occurred either without prodromal symptoms or were preceded by several days of insomnia, restlessness, irritability, or anxiety	Tobacco and alcohol use were considered by the study authors to be aggravating factors
Kaplan et al. (1965) 5 males among 26 workers (April–July 1962) Manufacturing	Inhalation, ingestion, and possible skin absorption as a result of the release of RDX dust into the workroom air during the dumping of dried RDX powder, screening and blending, and cleanup of spilled material without adequate ventilation	Sudden convulsions or loss of consciousness without convulsions; few or no premonitory symptoms (e.g., headache, dizziness, nausea, vomiting); stupor, disorientation, nausea, vomiting, and weakness; no changes in complete blood counts or urinalysis	Mild cases of RDX intoxication may have been masked by viral illness with nonspecific symptoms (e.g., headache, weakness, upset gastrointestinal [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 blood urea nitrogen [BUN]); liver or muscle damage (high aspartate transaminase [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)
Stone et al. (1969) 4 males (March–December 1968) Wartime, Vietnam	Ingestion of 180 g (patient 1), or 25 g (patients 2, 3) of C-4 (91% RDX)	Generalized seizures, lethargy, nausea, vomiting, fever, muscle soreness, headaches, twitching, (semi)comatose, headaches, hematuria, abnormal laboratory findings, muscle injury, elevated AST; no kidney damage For the patient who ingested the highest dose, anemia and loss of recent memory present after 30 d	Troops ingested small quantities of RDX to get a feeling of inebriation similar to that induced by ethanol

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Hollander and Colbach (1969) 5 males (June 1968–January 1969) Wartime, Vietnam	Inhalation (all 5 cases) and ingestion of unknown quantity of C-4 (2 cases)	Tonic-clonic seizures; nausea and vomiting occurred before and after admission; hyperirritability, muscle twitching, convulsions, mental confusion, and amnesia; kidney damage (oliguria, gross hematuria, proteinuria, elevated BUN); liver or muscle damage (high AST), leukocytosis; symptoms cleared by the next day except for amnesia (in case 2), oliguria (lasted for 4 d), and gross hematuria (decreased by 9th hospital day)	
Knepshield and Stone (1972) 6 males Wartime, Vietnam	Ingestion of C-4, range 25–180 g, average 77 g	Generalized seizures, coma, lethargy, severe neuromuscular irritability with twitching and hyperactive reflexes, myalgia, headache, nausea, vomiting, oliguria, gross hematuria, low-grade fever; abnormal laboratory findings (neutrophilic leukocytosis, azotemia, elevated AST)	Includes data on 2 patients from Merrill (1968)
Ketel and Hughes (1972) 18 males (December 1968–December 1969) Wartime, Vietnam	Inhalation while cooking with C-4 and possible ingestion	Central nervous system signs: confusion, marked hyperirritability, involuntary twitching of the extremities, severe prolonged generalized seizures, prolonged postictal mental confusion and amnesia, renal (oliguria and proteinuria; one case of acute renal failure requiring hemodialysis) and GI toxicity (nausea, vomiting)	C-4 was cut with the same knife used to stir/prep 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; electroencephalogram (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 hr; within 24 hr 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

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Goldberg et al. (1992) 1 male Nonwartime	Ingestion after chewing a piece (unknown size) of "Semtex" plastic explosive 4 hr before first seizure	Frontal headache and two tonic-clonic seizures; progressively disseminating petechial rash suggestive of meningococcal infection apyrexial; normotensive; no photophobia; no neurological abnormalities; florid petechial rash over the face and trunk; lacerated tongue. Initial results included: leukocyte count of 10.8×10^9/dl (87% neutrophils); hemoglobin, platelet count, coagulation screen, serum and cerebrospinal fluid (CSF) biochemistry all within normal limits; CSF and blood bacteriologically unremarkable. Shortly following admission, his headache and rash disappeared. There were no further seizures	
Harrell-Bruder and Hutchins (1995) 1 male Nonwartime	Ingestion of C-4 (chewing on a piece of undetermined size)	Tonic-clonic seizures, postictal state, EEGs were normal; brisk deep tendon reflexes	
Testud et al. (1996b) 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. (1996a) 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	

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
Küçükardali et al. (2003) 5 males Nonwartime	Ingestion (accidental) of 37−250 mg/kg body weight RDX during military training via food contaminated with RDX	Abdominal pain, nausea, vomiting, myalgia, headache, generalized weakness, repetitive tonic-clonic convulsions, lethargic or comatose between seizures, hyperactive deep tendon reflexes, sinusal tachycardia. Elevated serum levels of AST and ALT. Kidney damage was observed. Plasma RDX levels 3 hr after ingestion ranged from 268 to 969 pg/mL.	
Davies et al. (2007) 17 males Nonwartime	Ingestion of unknown quantity C-4 under unclear circumstances, but unrelated to recreational abuse	Seizures, headache, nausea and vomiting; hypokalemia and elevated creatine kinase, lactate dehydrogenase, and phosphate were noted in all but two patients. Metabolic acidosis only occurred in two patients directly following seizures	Patient histories may have been affect 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 white blood cell count and elevated creatine phosphokinase). Proteinuria and gross hematuria were observed.	

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Table C-10. Epidemiologic studies of RDX: summary of methodologic features

Reference, setting and design	Participants, selection, follow-up	Consideration of likely selection bias	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results details	Sample size
Occupational exposure studies							
Ma and Li (1992) (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, NR.	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 (SD)], 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, block design.	No adjustment for other risk factors, e.g., alcohol consumption. No consideration or attempt to distinguish TNT.	Comparisons of mean scaled score on memory retention, letter cancellation, or block design test; mean time on reaction tests; and total behavioral score. Variance (F test), linear and multiple regression, and correlation analysis.	60 exposed; Group A (n = 30; 26 males, 4 females); Group B (n = 30); 32 referents (27 males, 5 females).
Hathaway and Buck (1977); (United States) Ammunition workers	2022 workers (1,017 exposed to open explosives (TNT, RDX, HMX), 1,005 referents) at five U.S. Army ammunition plants (IA, IL, TN). Participation rate, 76%	Potential healthy worker effect.	Atmospheric samples of all operations with potential exposure to open explosives taken in 1975. Range: ND–1.57 mg/m^3. 70 exposed workers with RDX at >0.01 mg/m^3 [the LOD]; mean: 0.28 mg/m^3 [SD not	Liver function, renal function, and hematology tests [blood].	Workers with TNT exposure excluded from exposed groups.	Comparison of mean value; prevalence of elevated value on an individual test.	69 RDX exposed (43 males, 26 females), 24 RDX/HMX exposed (all males), 338 referents (237 males, 101 females).

Reference, setting and design	Participants, selection, follow-up	Consideration of likely selection bias	Exposure measure and range	Outcome measure	Consideration of likely confounding	Analysis and results details	Sample size
	exposed, 71% referents.		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 IH monitoring results.				
West and Stafford (1997); (United Kingdom) Ammunition workers (Case-control study)	378 of 404 subjects (excluded 3 deaths and 23 subjects with unknown addresses) previously studied in 1991, 32 cases with abnormal hematology test and 322 controls with normal hematology test. Participation rate among eligible subjects, 97% cases, 93% controls.	Former employees who were unable to work due to adverse health outcome were not included in the 1991 prevalence study.	Semiquantitative assessment; source of IH data not reported. Interviews with current and past employees and job title analysis to identify potential exposure to 100 agents, including RDX. Exposure surrogate was >50 hrs duration and intensity (low [1−10 ppm], moderate [10−100 ppm], and high [100−1,000 ppm]). RDX exposure	Abnormal hematology value in 1991 survey indicating possible meylodysplasia: neutropenia (2.0 x 10^9/l), low platelet count (<150 x 109/l), or macrocytosis (mean corpuscular volume = 99 fl or >6% macrocytes).	Cases and controls are not matched and statistical analyses are 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).

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
			prevalence (males), 83%.				

[a]Ma and Li (1992) describes symptoms reported by subjects during a physical examination but are not included in the evidence table because responses for individual symptoms were not identified.

ATSDR = Agency for Toxic Substances and Disease Registry, HMX = cyclotetramethylenetetranitramine, IA = Iowa, IL = Illinois, mg/m^3 = milligram/cubic meter, ND = not detected, SD = standard deviation, TN = Tennessee, TNT = trinitrotoluene

C.4. OTHER PERTINENT TOXICITY INFORMATION

Genotoxicity

RDX. RDX has tested negative in a variety of in vitro tests for genotoxicity, including mutation assays in multiple strains of Salmonella typhimurium (with or without metabolic activation), recombination in Saccharomyces cerevisiae strain D3, and forward mutations in both V79 Chinese hamster lung cells and mouse lymphoma L5178Y cells. However, in genotoxicity assays designed to be more sensitive, RDX did show some positive results. For example, when the concentration of S9 was doubled, the mutagenicity of RDX was about twice that of background. RDX also showed positive mutagenic results with metabolic activation in a chemiluminescent assay (Mutatox assay). In some cases, the interpretation of testing data for RDX was complicated by the tendency of the compound to precipitate out of DMSO solution (the usual vehicle) at concentrations ≥250 µg/mL (Reddy et al., 2005). As with other studies of RDX, the purity of the test compound was unknown in several (particularly older) studies. A summary of the results of in vitro genotoxicity studies of RDX is presented in Table C-11.

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; Minor et al., 1982; Cholakis et al., 1980; Whong et al., 1980; Simmon et al., 1977a). 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 (Simmon et al., 1977a). The study authors noted that the negative findings should be considered in the context of the low concentrations tested. Similarly, RDX did not induce forward mutations (HGPRT locus) in V79 Chinese hamster lung cells, with or without metabolic activation, although minimal cytotoxicity was observed at 180 µM (Lachance et al., 1999). However, RDX produced revertants in two of three trials in the Mutatox assay with the bacterium V. fisheri when tested at doses up to 2.5 µg/tube, with and without S9 (Arfsten et al., 1994). In the presence of S9, a dose-response was observed; in the absence of S9, no dose-response relationship was detected (Arfsten et al., 1994). RDX did not induce forward mutations in mouse lymphoma L5178Y cells with or without metabolic activation (Reddy et al., 2005). During an accompanying range-finding study, precipitates occurred at doses ≥250 µg/mL, suggesting that concentrations of RDX in DMSO reported beyond 250 µg/mL may not be accurate.

RDX did not induce unscheduled DNA synthesis, with or without metabolic activation, using human diploid fibroblasts (WI-38 cells) when tested in DMSO at concentrations up to 4,000 µg/mg;

however, precipitation of RDX at high concentrations in cell culture media makes interpretation of these results difficult (Dilley et al., 1979). Only two in vivo studies are available for the genotoxicity of RDX; these are summarized in Table C-12. RDX did not decrease the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE), 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 the number of live or dead embryos (Cholakis et al., 1980).

Metabolites of RDX. Several metabolites of RDX, N-nitroso derivatives of the parent compound (mononitroso, dinitroso, and trinitroso compounds, abbreviated MNX, DNX, and TNX, respectively) (Musick et al., 2010; Major et al., 2007), have been tested directly for genotoxicity (Pan et al., 2007a; George et al., 2001; Snodgrass, 1984). Miniature pigs were used to detect these trace metabolites because the swine model of the GI tract more closely resembles that of humans (Major et al., 2007); an identification and quantification of RDX metabolites in humans has not been conducted. A summary of the results of in vitro and in vivo genotoxicity studies of metabolites of RDX is presented in Table C-13.

Pan et al. (2007a) studied the mutagenicity of two metabolites, MNX and TNX. These metabolites were not mutagenic in TA97a at normal levels of S9 but clearly mutagenic at enhanced concentrations of S9 (4% versus 9% S9). The observation that these metabolites were positive in 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 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).

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

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

1 **Table C-11. Summary of in vitro studies of the genotoxicity of RDX**

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100	1,000 µg/plate	–	–	Metabolic activation with S9	Cholakis et al. (1980)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538 TA100, TA98	14 µg/plate	–	–	Effect of disinfection treatments on mutagenicity tested: RDX was not mutagenic in any strain before or after disinfection treatment with chlorine or ozone	Simmon et al. (1977b)
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	*Vibrio fischeri*	0.004 µg/tube	±	+	Mutatox assay with metabolic activation (S9)	Arfsten et al. (1994)
Reverse mutation (*umu* test)	*Salmonella choleraesius subsp. chol.* (prior *Salmonella 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)

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
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	*S. 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. (1977b)
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)

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

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

Table C-12. Summary of in vivo studies of the genotoxicity of RDX

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

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

Endpoint	Test system	Dose/ concentration[a]	Results[b]		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA97a, TA102	22 µg/plate	–	+	Mono and trinitroso metabolites (MNX and TNX); high S9 activation (9%) used	Pan et al. (2007a)
Reverse mutation	*S. typhimurium TA1535, TA1537, TA1538, TA98, TA100*	500 µg/plate	+	+	Positive 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.
[b]+ = positive; ± = equivocal or weakly positive; – = negative.

ND = not determined, NR = not reported

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

This appendix provides technical detail on dose-response evaluation and determination of points of departure (POD) for relevant toxicological endpoints. The endpoints were modeled using the EPA's Benchmark Dose Software (BMDS, Versions 2.4). Sections D.1 (noncancer) and D.2**Error! Reference source not found.** (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). In some cases it may be appropriate to use alternative methods, based on statistical judgement; exceptions are noted as necessary in the summary of the modeling results.

D.1. BENCHMARK DOSE MODELING SUMMARY FOR NONCANCER ENDPOINTS

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

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

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

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

[a]For convulsions in Crouse et al. (2006), the incidence rates across doses were determined to be not statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for this endpoint prior to modeling.
[b]The high dose group was excluded from modeling because a large proportion (approximately half) of the mice in that group died before wk 11, when the dose was reduced from 175 to 100 mg/kg-d.

D.1.1. Evaluation of Model Fit

For each dichotomous endpoint, BMDS dichotomous models[4] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.10 indicates lack of fit). Other factors were also used to assess model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the vicinity of the BMR.

D.1.2. Model Selection

For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is,

[4]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.

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

D.1.3. Modeling Results

Below are tables summarizing the modeling results for the noncancer endpoints modeled.

Nervous System Effects

Tables D-2 to D-10 present the BMD model results for incidence of convulsions for female, male, and male and female F344 rats combined based on data from Crouse et al. (2006), using BMRs of 10%, 5%, and 1% extra risk. Tables D-11 to D-13 present the BMD model results for incidence of convulsions for female F344 rats based on data from Cholakis (1980), using BMRs of 10%, 5%, and 1% extra risk.

Table D-2. Model predictions for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg/d)	$BMDL_{10Pct}$ (mg/kg/d)	Basis for model selection
	p-value	AIC			
Gamma	0.923	55.085	6.46	2.92	The quantal-linear model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.733	56.607	6.76	4.75	
LogLogistic	0.929	55.076	6.42	3.04	
Probit	0.793	56.086	6.64	4.54	
LogProbit	0.952	54.798	6.54	3.39	
Weibull	0.892	55.420	6.16	2.62	
Multistage 2°	0.954	53.595	5.46	2.47	
Quantal-Linear	**0.733**	**56.131**	**2.76**	**1.84**	
Multistage 5°[b]	0.885	55.525	5.98	2.47	
Multistage 4°	0.885	55.525	5.98	2.47	
Multistage 3°	0.885	55.525	5.98	2.49	
Dichotomous-Hill	0.964	56.265	7.10	5.75	

[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, −1.29, −0.46, −0.12, 0.87, and 0.41, respectively.

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

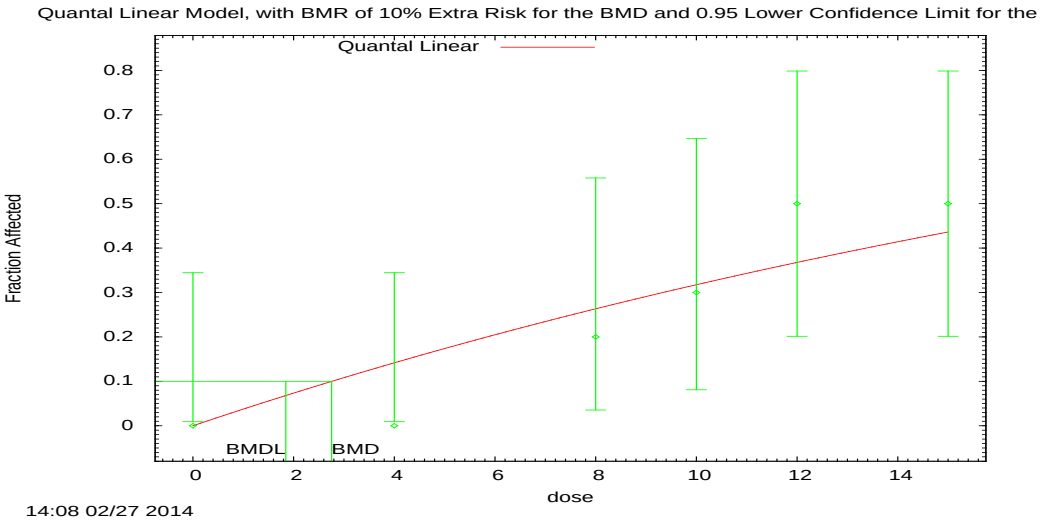

Figure D-1. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 10% extra risk; dose shown in mg/kg-day.

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 = 2.75544
BMDL at the 95% confidence level = 1.84489

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0.0833333
Slope	0.0382372	0.0404091
Power	n/a	1

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−24.9756	6			
Fitted model	−27.0654	1	4.17949	5	0.5239
Reduced model	−33.7401	1	17.529	5	0.003598

AIC: = 56.1307

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.1418	1.418	0	10	−1.286
8	0.2635	2.635	2	10	−0.456
10	0.3178	3.178	3	10	−0.121
12	0.368	3.68	5	10	0.866
15	0.4365	4.365	5	10	0.405

Chi^2 = 2.79 d.f = 5 P-value = 0.7325

Table D-3. Model predictions for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR= 5% extra risk

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.923	55.085	5.09	1.54	The quantal-linear model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.733	56.607	4.76	2.83	
LogLogistic	0.929	55.076	4.99	1.71	
Probit	0.793	56.086	4.80	2.69	
LogProbit	0.952	54.798	5.33	2.17	
Weibull	0.892	55.420	4.55	1.30	
Multistage 2°	0.954	53.595	3.81	1.21	
Quantal-Linear	**0.733**	**56.131**	**1.34**	**0.898**	
Multistage 3°	0.885	55.525	4.30	1.21	
Multistage 4°[b]	0.885	55.525	4.30	1.21	
Multistage 5°	0.885	55.525	4.30	1.20	
Dichotomous-Hill	0.964	56.265	6.25	2.30	

[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, −1.29, −0.46, −0.12, 0.87, and 0.41, respectively. The BMD_{10} and $BMDL_{10}$ for the selected model were 2.76 and 1.84 mg/kg-d, respectively.

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

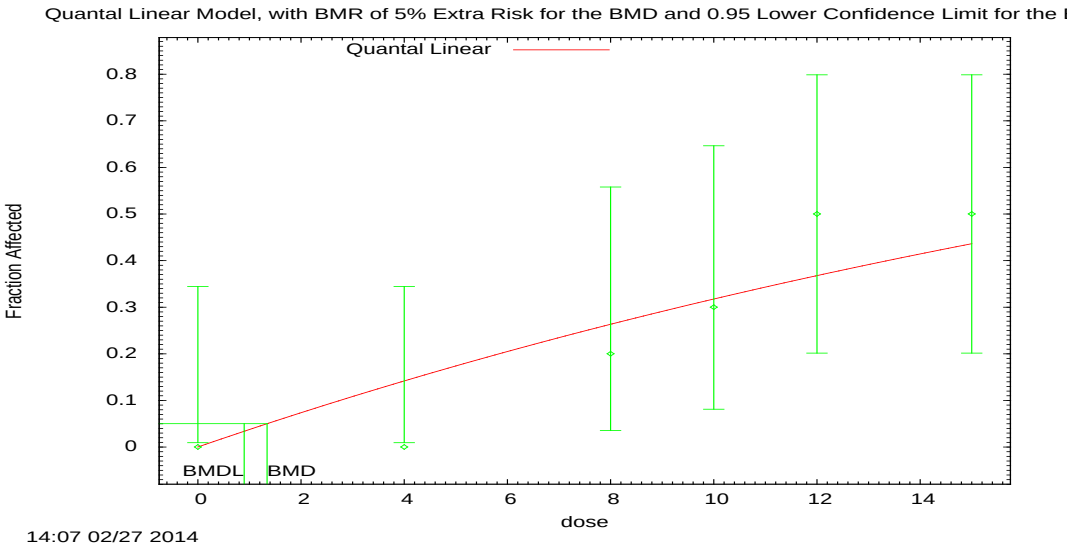

Figure D-2. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR= 5% extra risk; dose shown in mg/kg-day.

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 = 1.34145

BMDL at the 95% confidence level = 0.89816

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0.0833333
Slope	0.0382372	0.0404091
Power	n/a	1

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−24.9756	6			
Fitted model	−27.0654	1	4.17949	5	0.5239
Reduced model	−33.7401	1	17.529	5	0.003598

AIC: = 56.1307

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.1418	1.418	0	10	−1.286
8	0.2635	2.635	2	10	−0.456
10	0.3178	3.178	3	10	−0.121
12	0.368	3.68	5	10	0.866
15	0.4365	4.365	5	10	0.405

Chi^2 = 2.79 d.f = 5 P-value = 0.7325

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

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

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-d were 0.00, −0.67, 0.14, 0.11, 0.64, and −0.51, respectively. The BMD_{10} and $BMDL_{10}$ for the selected model were 5.98 and 2.47, respectively.

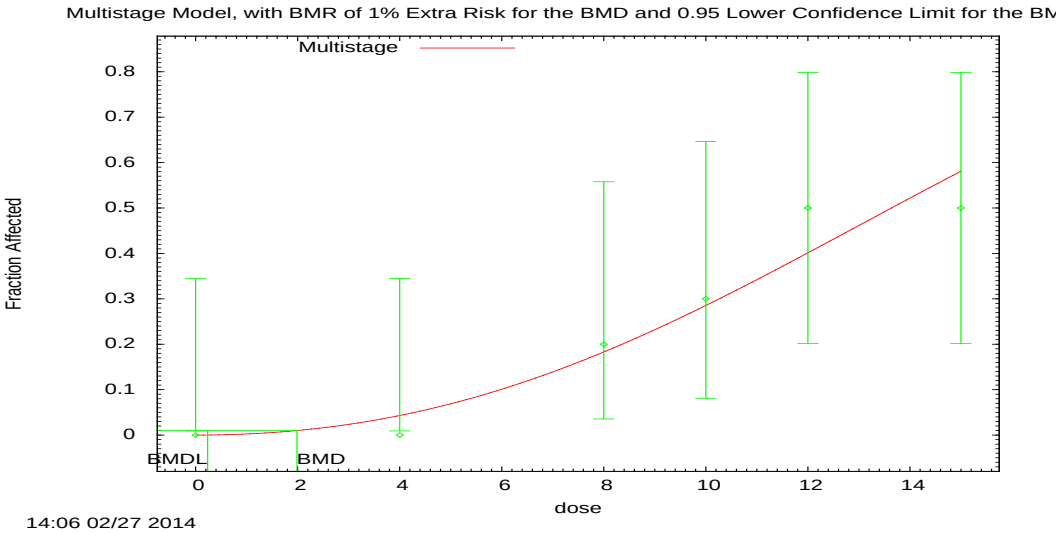

Figure D-3. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR= 1% extra risk; dose shown in mg/kg-day.

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 = 1.98616
BMDL at the 95% confidence level = 0.235433

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.0172961
Beta(2)	0.00234798	0.002476
Beta(3)	0.000100566	0
Beta(4)	0	0
Beta(5)	0	0

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−24.9756	6			
Fitted model	−25.7624	2	1.57351	4	0.8135
Reduced model	−33.7401	1	17.529	5	0.003598

AIC: = 55.5247

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.043	0.43	0	10	−0.671
8	0.1827	1.827	2	10	0.141
10	0.2849	2.849	3	10	0.106
12	0.4006	4.006	5	10	0.641
15	0.5801	5.801	5	10	−0.513

Chi^2 = 1.16 d.f = 4 P-value = 0.8854

Table D-5. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.482	48.534	7.40	5.11	Of the models that provided an adequate fit, the multistage 3° model was selected based on lowest AIC.
Logistic	0.335	49.692	7.18	5.03	
LogLogistic	0.522	48.248	7.48	5.29	
Probit	0.363	49.460	7.17	4.86	
LogProbit	0.530	48.224	7.53	5.38	
Weibull	0.376	49.496	6.84	4.47	
Multistage 2°	0.307	50.335	4.54	2.95	
Quantal-Linear	0.0553	56.530	1.98	1.38	
Multistage 5°[b] Multistage 4°	0.361	49.607	6.85	3.91	
Multistage 3°	**0.515**	**47.803**	**6.17**	**3.95**	
Dichotomous-Hill	0.701	48.408	8.34	6.32	

[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, −0.54, −0.82, −0.40, 1.66, and −0.61, respectively.

[b]For the Multistage 5° model, the b4 coefficient estimates was 0 (boundary of parameters space). The models in this row reduced to the Multistage 4° model.

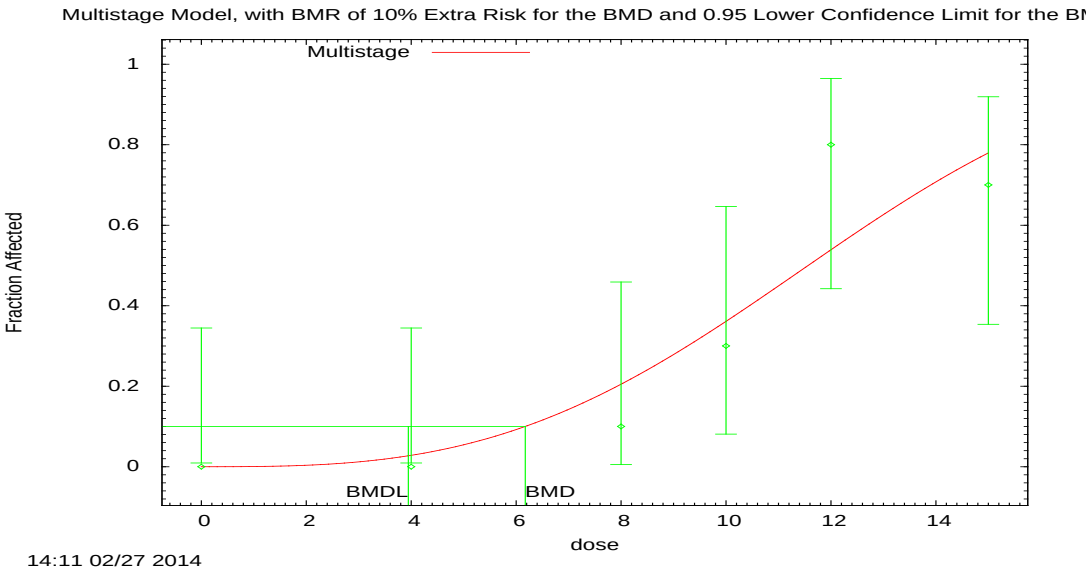

Figure D-4. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 10% extra risk; dose shown in mg/kg-day.

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 = 10% Extra risk

BMD = 6.17392

BMDL at the 95% confidence level = 3.94501

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0
Beta(2)	0	0.00691555
Beta(3)	0.000447707	0

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−20.4721	6			
Fitted model	−22.9013	1	4.85838	5	0.4334
Reduced model	−37.4599	1	33.9755	5	<0.0001

AIC: = 47.8027

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.0282	0.282	0	10	−0.539
8	0.2049	2.049	1	10	−0.822
10	0.3609	3.609	3	10	−0.401
12	0.5387	5.387	8	10	1.658
15	0.7793	7.793	7	10	−0.605

Chi^2 = 4.24 d.f = 5 P-value = 0.5153

Table D-6. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 5% extra risk

Model[a]	Goodness of fit		BMD_{5Pct} (mg/kg-d)	$BMDL_{5Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.482	48.534	6.47	3.96	Of the models that provided an adequate fit, the multistage 3° model was selected based on lowest AIC.
Logistic	0.335	49.692	5.74	3.34	
LogLogistic	0.522	48.248	6.51	4.14	
Probit	0.363	49.460	5.92	3.26	
LogProbit	0.530	48.224	6.71	4.40	
Weibull	0.376	49.496	5.58	3.16	
Multistage 2°	0.307	50.335	3.17	1.63	
Quantal-Linear	0.0553	56.530	0.964	0.670	
Multistage 5°[b] Multistage 4°	0.361	49.607	5.56	1.99	
Multistage 3°	**0.515**	**47.803**	**4.86**	**2.19**	
Dichotomous-Hill	0.701	48.408	7.76	5.27	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-d were 0.000, −0.54, −0.82, −0.40, 1.66, and −0.61, respectively. The BMD_{10} and $BMDL_{10}$ for the selected model were 6.17 and 3.95 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.

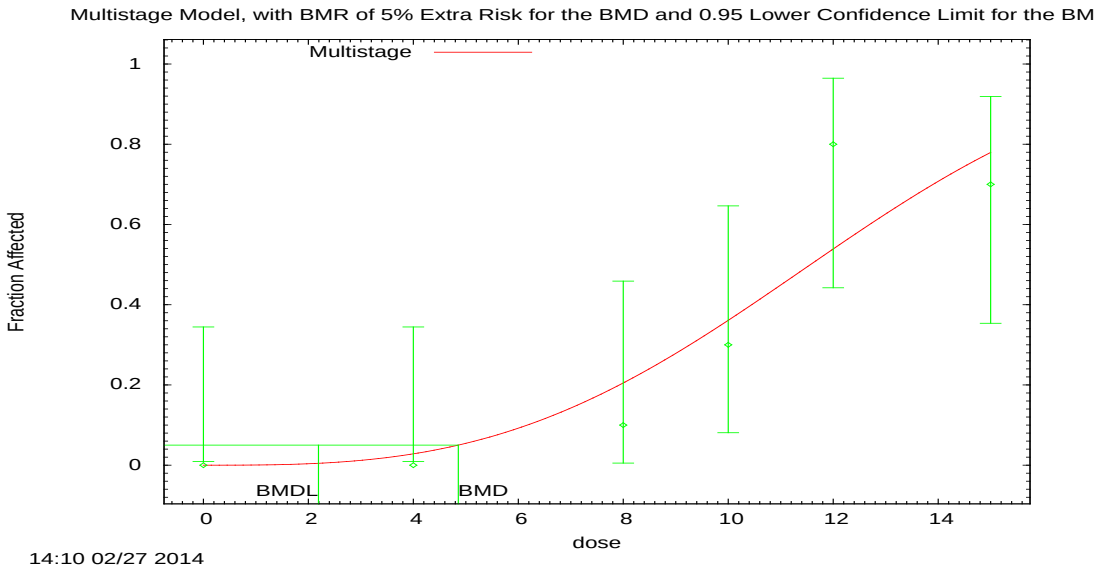

Figure D-5. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 5% extra risk; dose shown in mg/kg-day.

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 = 5% Extra risk

BMD = 4.85686

BMDL at the 95% confidence level = 2.19244

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0
Beta(2)	0	0.00691555
Beta(3)	0.000447707	0

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−20.4721	6			
Fitted model	−22.9013	1	4.85838	5	0.4334
Reduced model	−37.4599	1	33.9755	5	<0.0001

AIC: = 47.8027

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	10	0
4	0.0282	0.282	0	10	−0.539
8	0.2049	2.049	1	10	−0.822
10	0.3609	3.609	3	10	−0.401
12	0.5387	5.387	8	10	1.658
15	0.7793	7.793	7	10	−0.605

Chi^2 = 4.24 d.f = 5 P-value = 0.5153

Table D-7. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% extra risk

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

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

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

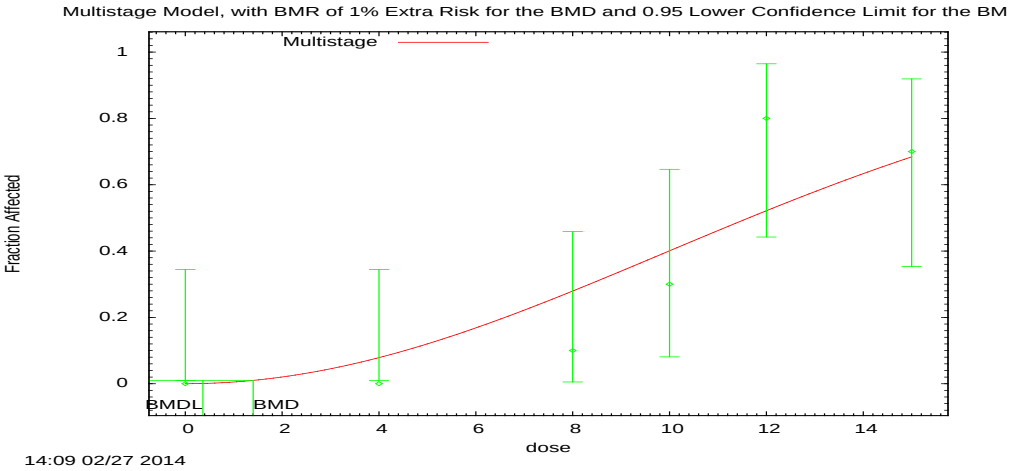

Figure D-6. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% extra risk; dose shown in mg/kg-day.

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 = 1.40125
BMDL at the 95% confidence level = 0.363499

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 50.3345

Goodness of Fit Table

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

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

Table D-8. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.484	101.79	6.92	5.09	Of the models that provided an adequate fit, the multistage 3° model was selected based on lowest AIC.
Logistic	0.231	104.55	6.86	5.34	
LogLogistic	0.512	101.66	6.93	5.15	
Probit	0.291	103.61	6.83	5.19	
LogProbit	0.557	101.25	7.01	5.31	
Weibull	0.369	102.91	6.52	4.62	
Multistage 2°	0.364	103.03	4.97	3.75	
Quantal-Linear	0.0369	111.56	2.32	1.77	
Multistage 3°[b] **Multistage 4°** **Multistage 5°**	**0.502**	**100.91**	**6.60**	**4.59**	
Dichotomous-Hill	0.696	101.64	7.73	5.98	

[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, −0.69, −0.25, −0.06, 1.62, and −1.08, respectively.

[b]For the Multistage 4° and 5° models, the b4 and b5 coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 3° model.

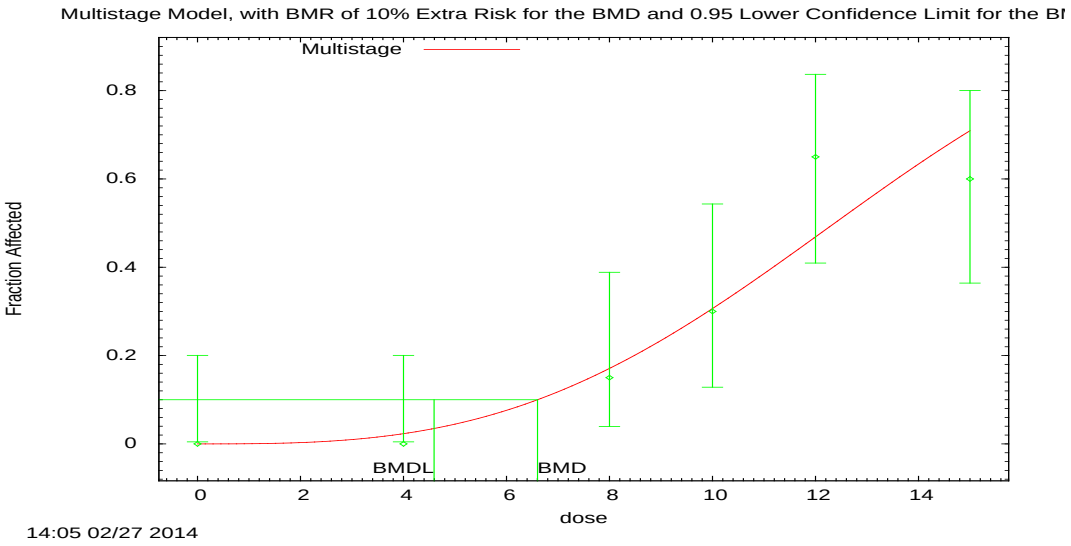

Figure D-7. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 10% extra risk; dose shown in mg/kg-day.

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 = 10% Extra risk

BMD = 6.60247

BMDL at the 95% confidence level = 4.59346

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 degrees of freedom	*p*-value
Full model	−47.0806	6			
Fitted model	−49.4567	1	4.75213	5	0.4469
Reduced model	−71.5289	1	48.8965	5	<0.0001

AIC: = 100.913

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.0232	0.463	0	20	−0.689
8	0.1709	3.418	3	20	−0.248
10	0.3065	6.131	6	20	−0.063
12	0.4688	9.375	13	20	1.624
15	0.7093	14.186	12	20	−1.076

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

Table D-9. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 5% extra risk

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	Of the models that provided an adequate fit, the multistage 3° model was selected based on lowest AIC.
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 2°	0.364	103.03	3.47	2.20	
Quantal-Linear	0.0369	111.56	1.13	0.860	
Multistage 3°	**0.502**	**100.91**	**5.19**	**2.66**	
Multistage 4° Multistage 5°[b]	0.502	100.91	5.19	2.65	
Dichotomous-Hill	0.696	101.64	6.98	4.80	

[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, −0.69, −0.25, −0.06, 1.62, and −1.08, respectively. The BMD_{10} and $BMDL_{10}$ for the selected model were 6.60 and 4.59 mg/kg-d, respectively.

[b]For the Multistage 5° model, the b5 coefficient estimate was 0 (boundary of parameters space). The models in this row reduced to the Multistage 4° model.

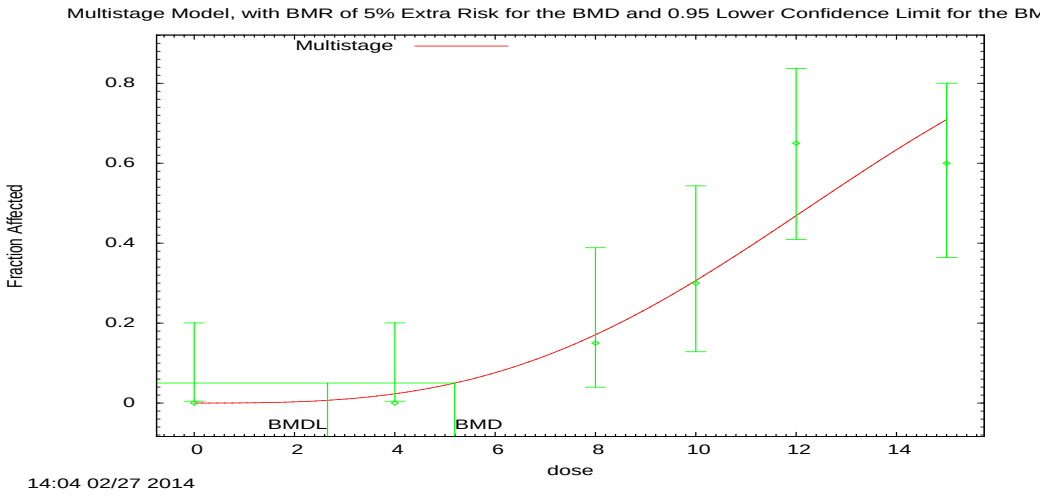

Figure D-8. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 5% extra risk; dose shown in mg/kg-day.

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 = 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 degrees of freedom	*p*-value
Full model	−47.0806	6			
Fitted model	−49.4567	1	4.75213	5	0.4469
Reduced model	−71.5289	1	48.8965	5	<0.0001

AIC: = 100.913

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.0232	0.463	0	20	−0.689
8	0.1709	3.418	3	20	−0.248
10	0.3065	6.131	6	20	−0.063
12	0.4688	9.375	13	20	1.624
15	0.7093	14.186	12	20	−1.076

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

Table D-10. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% extra risk

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

[a]Selected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-d were 0.00, −1.19, −0.93, −0.45, 1.71, and −0.16, respectively. The BMD_{10} and $BMDL_{10}$ for the selected model were 4.97 and 3.75 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.

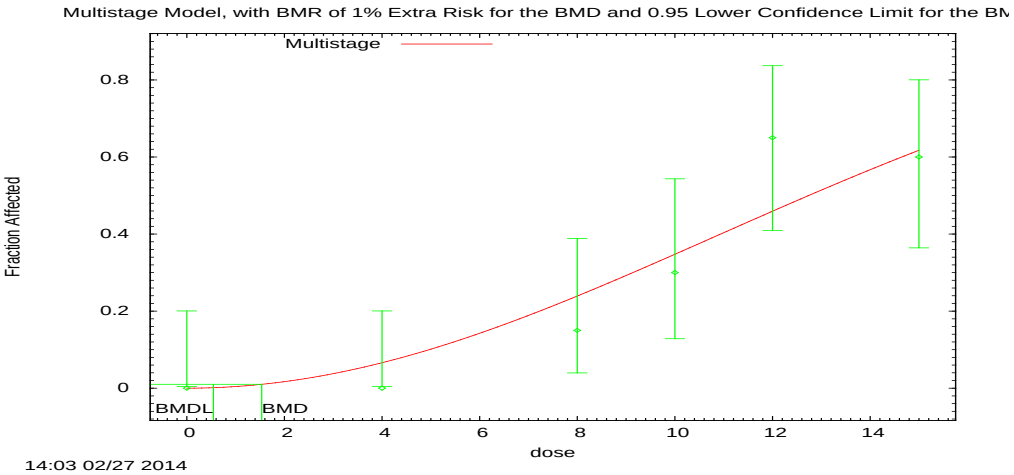

Figure D-9. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% extra risk; dose shown in mg/kg-day.

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 = 1.53434
BMDL at the 95% confidence level = 0.544329

Parameter Estimates

Variable	Estimate	Default initial parameter values
Background	0	0
Beta(1)	0	0.00163806
Beta(2)	0.00426912	0.00485933

Analysis of Deviance Table

Model	Log(likelihood)	Number of parameters	Deviance	Test degrees of freedom	*p*-value
Full model	−47.0806	6			
Fitted model	−50.5158	1	6.87034	5	0.2305
Reduced model	−71.5289	1	48.8965	5	<0.0001

AIC: = 103.032

Goodness of Fit Table

Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0	0	0	20	0
4	0.066	1.321	0	20	−1.189
8	0.2391	4.782	3	20	−0.934
10	0.3475	6.95	6	20	−0.446
12	0.4592	9.185	13	20	1.712
15	0.6173	12.346	12	20	−0.159

Chi^2 = 5.44 d.f = 5 P-value = 0.3644

Table D-11. Model predictions for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.989	42.003	3.62	1.56	The quantal-linear model is selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.526	43.556	8.92	6.14	
LogLogistic	0.991	41.996	3.45	1.53	
Probit	0.577	43.348	7.64	5.39	
LogProbit	1.000	41.963	3.13	1.51	
Weibull	0.983	42.026	3.81	1.55	
Multistage 3°[b]	0.960	42.113	4.26	1.54	
Multistage 2°	0.960	42.113	4.26	1.54	
Quantal-Linear	**0.669**	**42.077**	**1.88**	**1.29**	

[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.

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

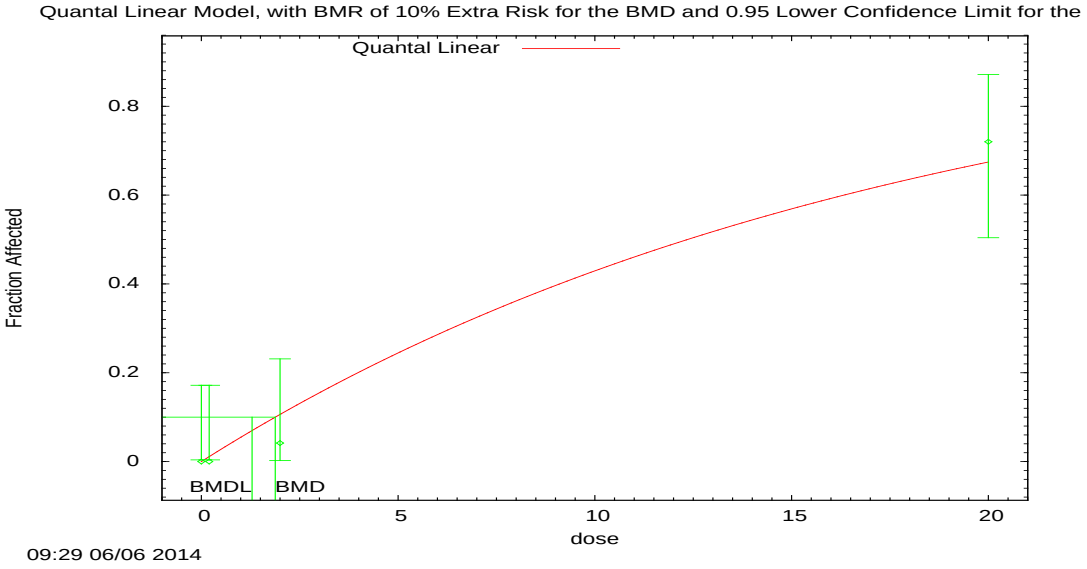

Figure D-10. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980). BMR = 10% extra risk; dose shown in mg/kg-day.

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 = 1.87886
BMDL at the 95% confidence level = 1.28909

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 degrees of freedom	*p*-value
Full model	−18.9808	4			
Fitted model	−20.0384	1	2.11537	3	0.5488
Reduced model	−47.9793	1	57.9972	3	<0.0001

AIC: = 42.0769

Goodness of Fit Table

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

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

Table D-12. Model predictions for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980); BMR = 5% extra risk

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	The quantal-linear model is 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 2°	0.960	42.113	2.51	0.747	
Quantal-Linear	**0.669**	**42.077**	**0.915**	**0.628**	
Multistage 3°[b]	0.960	42.113	2.51	0.747	

[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 $BMDL_{10}$ for the selected model were 1.88 and 1.29 mg/kg-d, respectively.

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

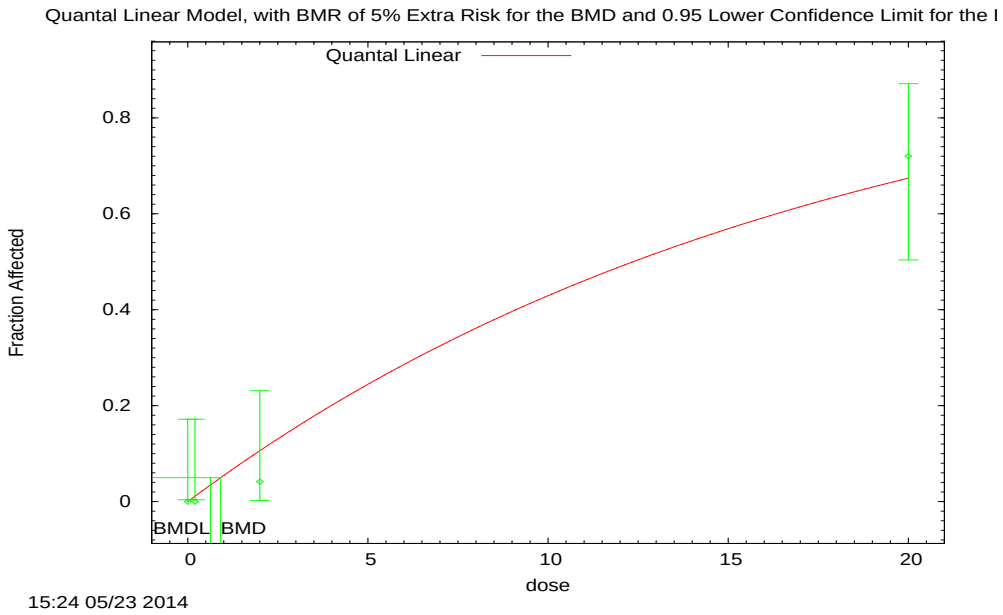

Figure D-11. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980). BMR = 5% extra risk; dose shown in mg/kg-day.

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 degrees of freedom	*p*-value
Full model	−18.9808	4			
Fitted model	−20.0384	1	2.11537	3	0.5488
Reduced model	−47.9793	1	57.9972	3	<0.0001

AIC: = 42.0769

Goodness of Fit Table

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

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

Table D-13. Model predictions for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980); BMR = 1% extra risk

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

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

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

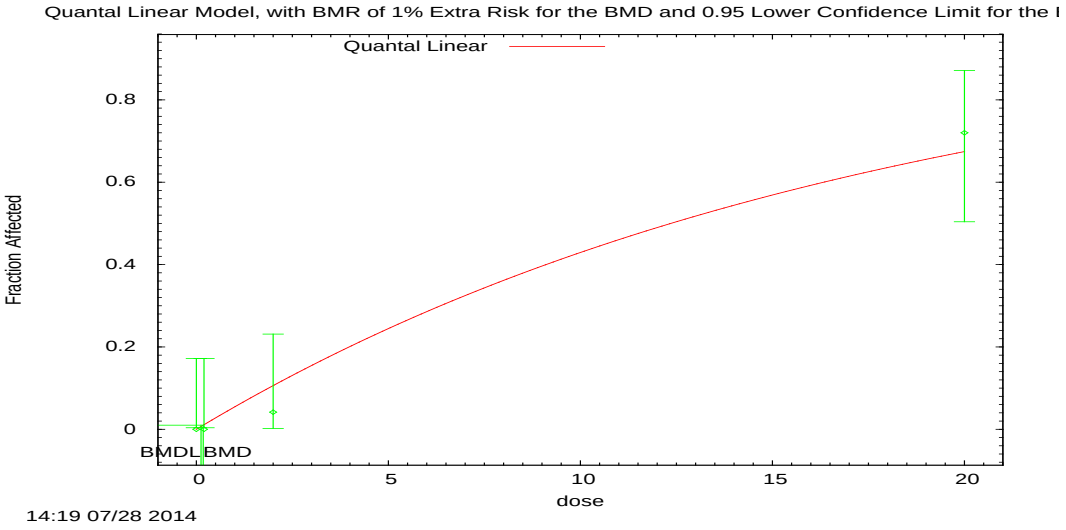

Figure D-12. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in female F344 rats exposed to RDX by gavage on gestation days 6–19 (Cholakis, 1980). BMR = 1% extra risk; dose shown in mg/kg-day.

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 = 1% Extra risk
BMD = 0.179224
BMDL at the 95% confidence level = 0.122966

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 42.0769

Goodness of Fit Table

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

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

Male Reproductive Effects

Table D-14 presents the BMD model results for incidence of testicular degeneration for male B6C3F$_1$ mice based on data from Lish et al. (1984), using a BMR of 10% extra risk.

Table D-14. Model predictions for testicular degeneration in male B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% extra risk

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg-d)	$BMDL_{10Pct}$ (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma[b] Weibull Quantal-Linear	0.357	101.10	66.5	35.4	The log-probit model was selected based on lowest BMDL (BMDLs differed by more than threefold).
Logistic	0.159	103.40	97.1	66.1	
LogLogistic	0.377	100.91	63.6	32.3	
Probit	0.178	103.12	93.1	61.4	
LogProbit	**0.876**	**97.564**	**56.0**	**16.3**	
Multistage 2°[c] Multistage 3° Multistage 4°	0.357	101.10	66.5	35.4	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-d were 0.00, 0.32, −0.61, 0.43, and −0.17, respectively.

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

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

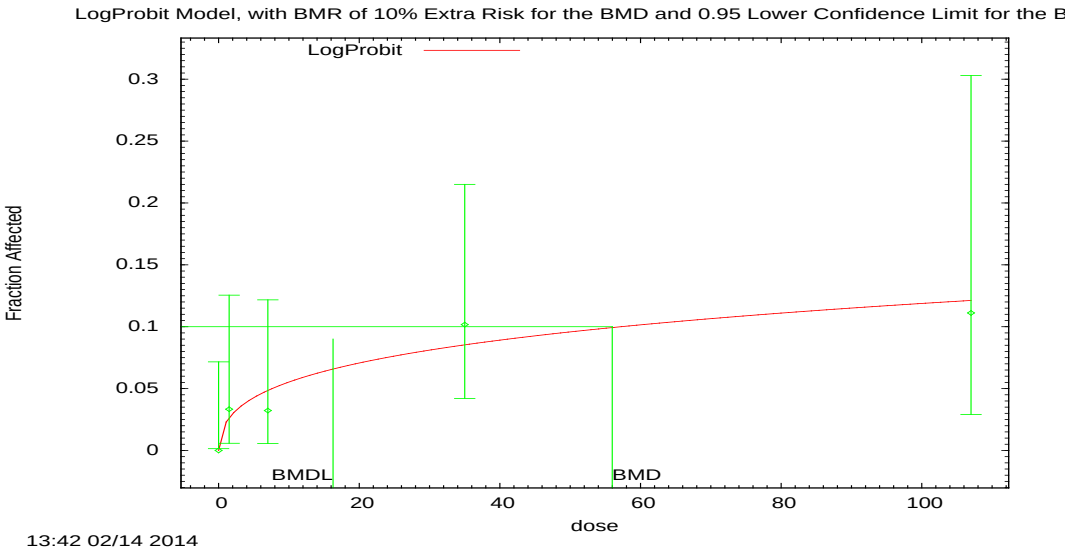

Figure D-13. Plot of incidence rate by dose, with fitted curve for selected model, for testicular degeneration in male B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984). BMR = 10% extra risk; dose shown in mg/kg-day.

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 = 55.9784

BMDL at the 95% confidence level = 16.2787

Parameter Estimates

Variable	Estimate	Default initial parameter values
background	0	0
intercept	−2.0054E+00	−1.9976E+00
slope	0.179828	0.172286

Analysis of Deviance Table

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

AIC: = 97.5635

Goodness of Fit Table

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

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

Kidney Effects

Table D-15 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% extra risk.

Table D-15. Model predictions for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 10% extra risk

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).
Logistic	0.102	203.50	10.8	8.58	
LogLogistic	0.328	200.05	3.33	2.09	
Probit	0.116	203.10	9.91	7.96	
LogProbit	**0.204**	**202.03**	**1.67**	**0.469**	
Weibull[g]	0.288	200.37	4.61	3.24	

[a]Selected model in bold; scaled residuals for selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-d were −0.289, 0.172, 0.846, −1.298, and 0.819, respectively.

[b]The Gamma model had a power parameter estimate was 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.

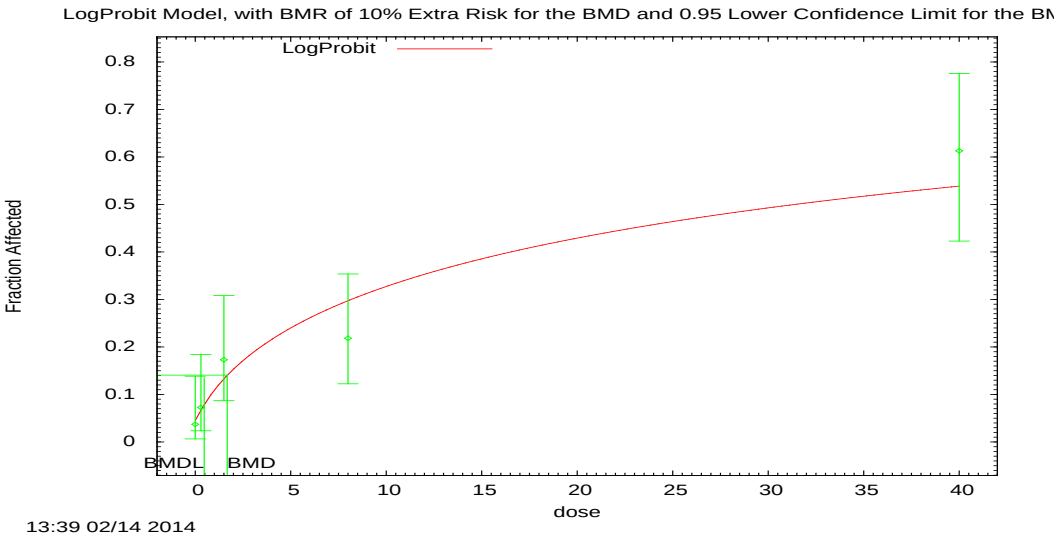

Figure D-14. Plot of incidence rate by dose, with fitted curve for selected model, for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983). BMR = 10% extra risk; dose shown in mg/kg/day.

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 degrees of freedom	*p*-value
Full model	−96.3905	5			
Fitted model	−98.0147	3	3.24837	2	0.1971
Reduced model	−118.737	1	44.6933	4	<0.0001

AIC: = 202.029

Goodness of Fit Table

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

Chi^2 = 3.18 d.f = 2 P-value = 0.2035

D.2. BENCHMARK DOSE MODELING SUMMARY FOR CANCER ENDPOINTS

The cancer endpoints that were selected for dose-response modeling are presented in Table D-16. For each endpoint, the doses and tumor incidence data used for the modeling are presented.

Table D-16. Cancer endpoints selected for dose-response modeling for RDX

Endpoint and reference	Species/sex	Dose (mg/kg-d)	Incidence/total
Hepatocellular adenomas or carcinomas Parker et al. (2006)	Female B6C3F$_1$ mice	0 1.5 7 35 107	1/67 (1%) 4/62 (6%) 5/63 (8%) 10 /64 (16%) 4/31 (13%)
Alveloar/bronchiolar adenomas or carcinomas Lish et al. (1984)	Female B6C3F$_1$ mice	0 1.5 7 35 107	7/65 (11%) 3/62 (5%) 8/64 (13%) 12/64 (19%) 7/31 (23%)
Hepatocellular adenomas or carcinomas Levine et al. (1983)	Male F344 rats	0 0.3 1.5 8 40	1/55 (2%) 0/55 (0%) 0/52 (0%) 2/55 (4%) 2/31[a] (6%)

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

D.2.1. Evaluation of Model Fit and Model Selection

For each endpoint, BMDS multistage-cancer models[5] were fitted to the data using the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square goodness-of-fit test (χ^2 p-value < 0.05[6] indicates lack of fit). Other factors were 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 BMR.

For each endpoint, the BMDL estimate (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC value were used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL estimates were "sufficiently close," that is, differed by more than threefold, the model selected was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest BMDL was selected as the POD.

After selecting models for the two endpoints, the results were combined using MS-COMBO in BMDS. This procedure analyzes the incidence of a tumor (adenoma or carcinoma) defined as

[5]The coefficients of the multistage-cancer models were restricted to be nonnegative (beta's ≥ 0).
[6]A significance level of 0.05 is used for selecting cancer models because the model family (multistage) is selected a priori (*Benchmark Dose Technical Guidance Document*, U.S. EPA, 2012).

present if either the hepatocellular or alveolar/bronchiolar tumor (or both) was present, and not present otherwise. The two endpoints were assumed to be independent.

D.2.2. Modeling Results

Details of the BMD modeling for mouse tumor data sets are provided in Tables D-17 to D-20 below. In addition, this appendix presents a quantitative dose-response analysis using rat liver tumor data and detailed BMD modeling results (see Table D-22). The analysis of rat liver tumor data and resulting candidate OSF is presented for comparison with other OSF estimates provided in Section 2.3.3 of the Toxicological Review.

Mouse Tumor Data – BMD Modeling Documentation

Table D-17. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% extra risk

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

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

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

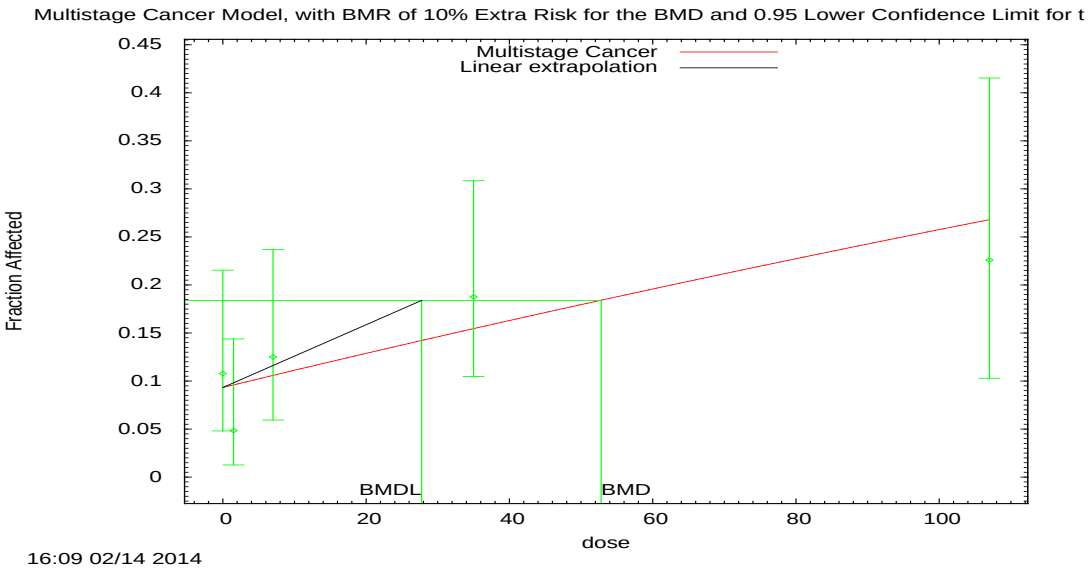

Figure D-15. Plot of incidence rate by dose, with the fitted curve for the selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984). BMR = 10% extra risk; dose shown in mg/kg-day.

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

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

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 10% Extra risk

BMD = 52.8078

BMDL at the 95% confidence level = 27.748

BMDU at the 95% confidence level = 194.806

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

Multistage Cancer Slope Factor = 0.00360387

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 218.682

Goodness of Fit Table

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

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

Table D-18. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 5% extra risk

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

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

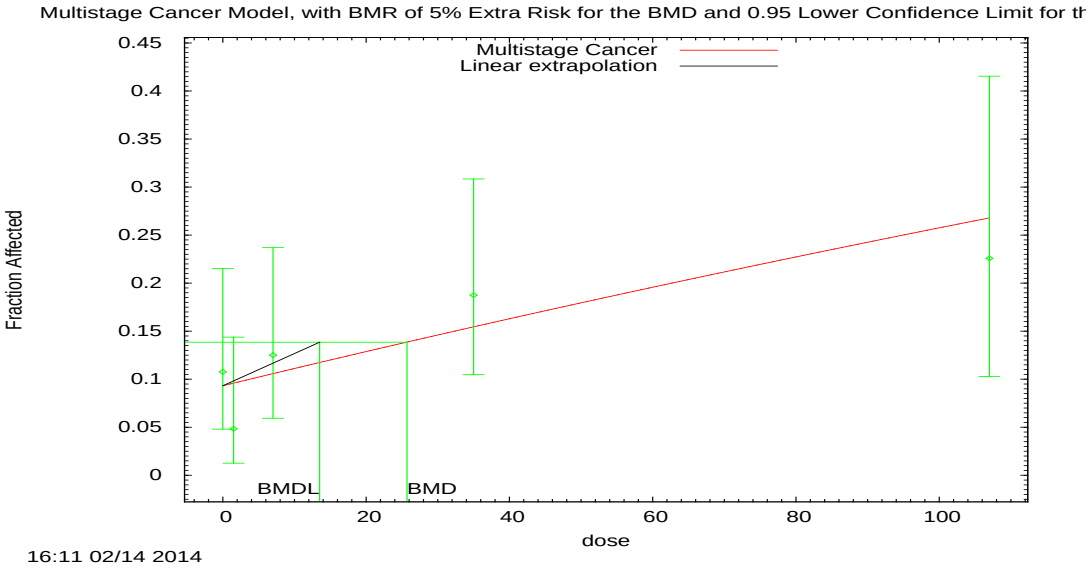

Figure D-16. Plot of incidence rate by dose, with fitted curve for selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Lish et al., 1984).
BMR = 5% extra risk; dose shown in mg/kg-day.

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

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

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 218.682

Goodness of Fit Table

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

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

Table D-19. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 10% extra risk

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

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

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

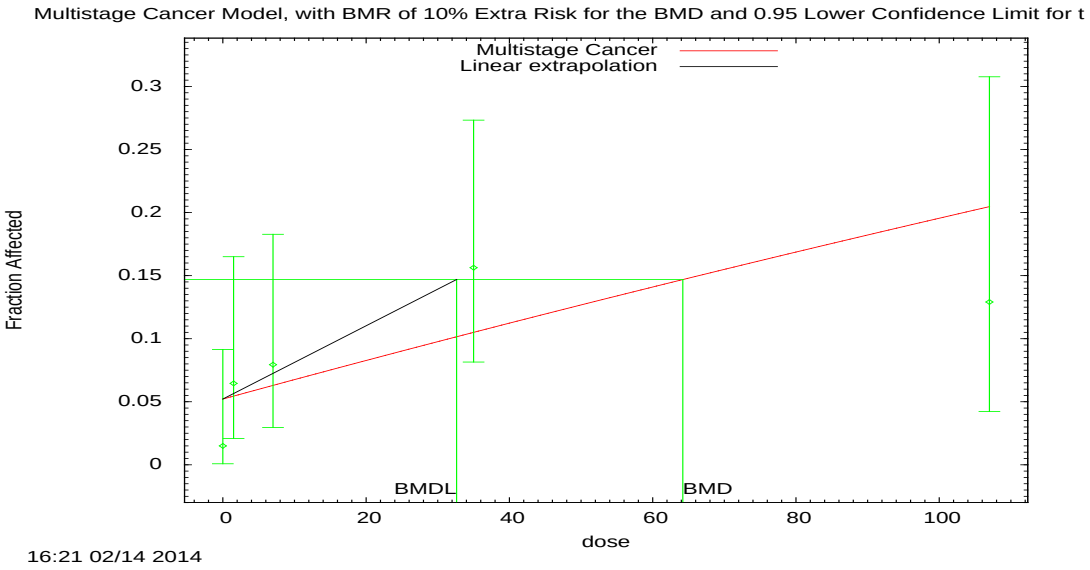

Figure D-17. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in female B6C3F$_1$ mice exposed to RDX by diet for 24 months (Parker et al., 2006). BMR = 10% extra risk; dose shown in mg/kg-day.

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

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

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 164.063

Goodness of Fit Table

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

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

Table D-20. Model predictions for B6C3F$_1$ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 5% extra risk

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°**	**0.160**	**164.06**	**31.3**	**15.9**	All the models reduced to the multistage 1° model, so it was selected.

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

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

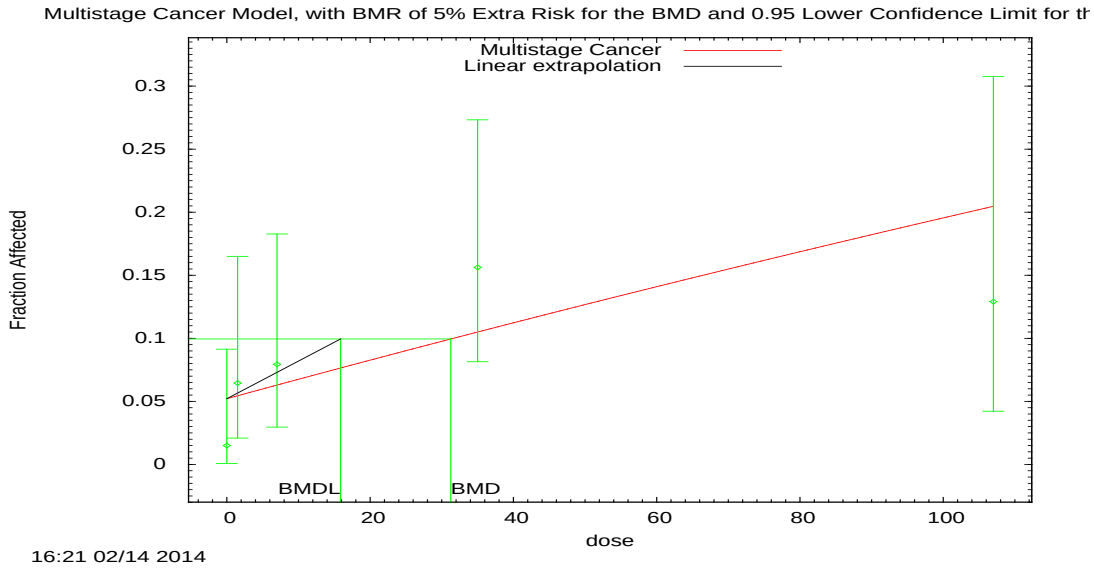

Figure D-18. Plot of incidence rate by dose, with fitted curve for selected model, for B6C3F$_1$ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006). BMR = 5% extra risk; dose shown in mg/kg-day.

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

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

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 164.063

Goodness of Fit Table

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

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

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

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

MSCOMBO results

BMR of 10% Extra Risk

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

 Combined Log-Likelihood           -187.3723596892213

 Combined Log-likelihood Constant       166.01737626058841


  Benchmark Dose Computation

Specified effect =      0.1

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    28.9753

      BMDL =    17.6574

Multistage Cancer Slope Factor =  0.00566334
```

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

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

MSCOMBO results

BMR of 5% Extra Risk

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

 Combined Log-Likelihood           -187.3723596892213

 Combined Log-likelihood Constant       166.01737626058841


  Benchmark Dose Computation

Specified effect =      0.05

Risk Type    =   Extra risk

Confidence level =      0.95

       BMD =    14.1062

      BMDL =    8.59627

Multistage Cancer Slope Factor =  0.00581647
```

Rat Tumor Data -- Dose-response Analysis and BMD Modeling Documentation

The incidence of liver carcinomas in male F344 rats from the study by Levine et al. (1983) was considered for quantitative dose-response analysis (see Table D-16) for comparison with other OSF estimates. The high-dose male group in Levine et al. (1983) had a markedly lower survival curve than the other dose groups, indicating a substantial number of early deaths in the high-dose group. In this case, a time-to-tumor analysis is preferred. Although tumor incidence was listed for each animal in this study, 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. Therefore, the times of death and the tumor incidence of the animals could not be matched, and a time-to-tumor analysis was not possible.

Tumor incidence was modeled using the multistage-cancer models in BMDS (version 2.5). Because the maximum liver tumor response in the male rat was 6.4%, a BMR of 5% was used to model male rat liver tumor data in order to obtain a BMD and BMDL in the range of the experimental data, as recommended in Section 3.2 of *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). To 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. These are the denominators listed in Table D-16.

To estimate the human equivalent dose at the BMDL, HEDs based on both administered dose scaled by $BW^{3/4}$ and PBPK modeling were considered. Confidence in the revised rat PBPK model is relatively high (see Appendix C, Section C.2.5); 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 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 are provided in Table D-21, and detailed BMD modeling results are provided in Table D-22. 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). Linear extrapoloation from the POD derived from these two dose-metrics resulted in candidate OSFs of 0.017 and 0.009 $(mg/kg\text{-}day)^{-1}$, respectively. It is important to note that EPA considered

that the association between RDX exposure and rat liver tumors is not strong, reflecting the relatively low magnitude of the rat liver carcinoma response and reduced confidence that the high-dose group accurately reflects lifetime cancer incidence because, in part, of low survival (see discussion in Section 1.1.5).

Table D-21. Model predictions and oral slope factor for hepatocellular carcinomas in male F344 rats administered RDX in the diet for 2 years (Levine et al., 1983)

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

[a]Slope factor = $BMR/BMDL_{05\text{-}HED}$, where BMR = 0.05 (5% extra risk).
[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-22. Model predictions for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 5% extra risk

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

[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.

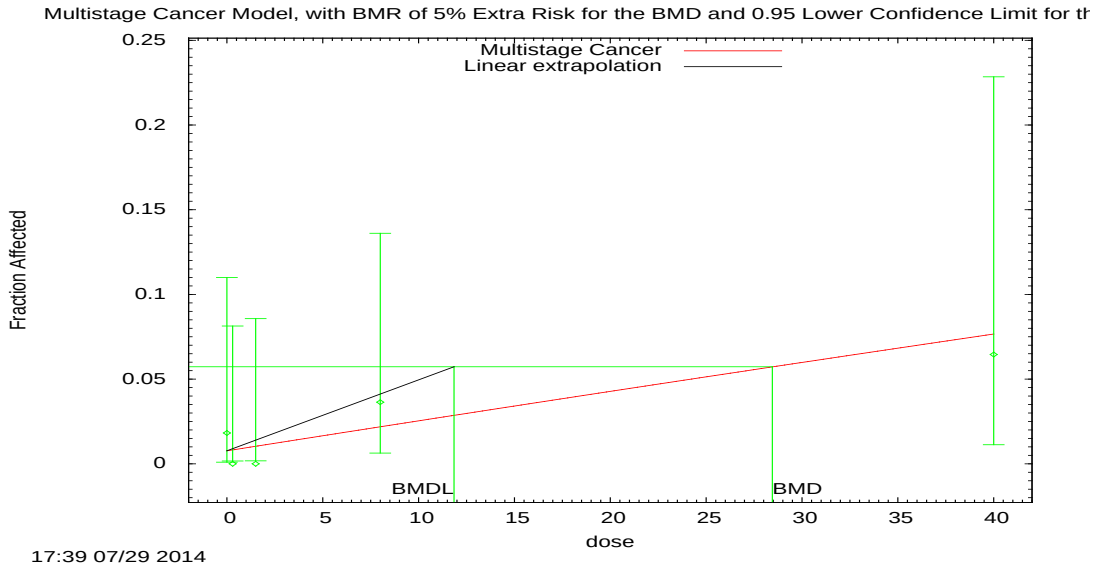

Figure D-19. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983). BMR = 5% extra risk; dose shown in mg/kg-day.

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 degrees of freedom	*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

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