



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

(CASRN 121-82-4)

Supplemental Information

September 2016

NOTICE

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

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

DISCLAIMER

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

CONTENTS

APPENDIX A.	ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES	A-1
APPENDIX B.	ADDITIONAL DETAILS OF LITERATURE SEARCH STRATEGY STUDY SELECTION AND EVALUATION	B-1
	B.1. DEFENSE TECHNICAL INFORMATION CENTER (DTIC) LITERATURE SEARCH AND SCREEN	B-1
APPENDIX C.	INFORMATION IN SUPPORT OF HAZARD IDENTIFICATION AND DOSE-RESPONSE ANALYSIS	C-1
	C.1. TOXICOKINETICS	C-1
	C.1.1. Absorption	C-1
	C.1.2. Distribution	C-6
	C.1.3. Metabolism	C-7
	C.1.4. Excretion	C-9
	C.1.5. Physiologically Based Pharmacokinetic (PBPK) Models	C-11
	C.2. HUMAN STUDIES	C-32
	C.3. OTHER PERTINENT TOXICITY INFORMATION	C-38
	C.3.1. Mortality in Animals	C-38
	C.3.2. Other Noncancer Effects	C-43
	C.3.3. Genotoxicity	C-63
APPENDIX D.	DOSE-RESPONSE MODELING FOR THE DERIVATION OF REFERENCE VALUES FOR EFFECTS OTHER THAN CANCER AND THE DERIVATION OF CANCER RISK ESTIMATES	D-1
	D.1. BENCHMARK DOSE MODELING SUMMARY FOR NONCANCER ENDPOINTS	D-1
	D.1.1. Evaluation of Model Fit and Model Selection	D-3
	D.1.2. Modeling Results	D-4
	D.1.3. Mortality: Dose-Response Analysis and BMD Modeling Documentation	D-20
	D.2. BENCHMARK DOSE MODELING SUMMARY FOR CANCER ENDPOINTS	D-30
	D.2.1. Evaluation of Model Fit and Model Selection for Mouse Tumor Data	D-31
	D.2.2. Modeling Results for Mouse Tumor Data	D-31

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

D.2.3. Dose-response Analysis and BMD Modeling Documentation for Other Tumor Data Sets	D-45
APPENDIX E. SUMMARY OF PUBLIC COMMENTS AND EPA’S DISPOSITION	E-1
REFERENCES FOR APPENDICES	R-1

TABLES

Table A-1. Assessments by other national and international health agencies.....	A-1
Table B-1. Summary of detailed search strategies for RDX (Pubmed, Toxline, Toxcenter, TSCATS).....	B-3
Table B-2. Summary of detailed search strategies for RDX (DTIC)	B-12
Table B-3. Processes used to augment the search of core databases for RDX.....	B-13
Table C-1. Distribution of RDX or radiolabel from administered RDX	C-7
Table C-2. Principal urinary metabolites of RDX in miniature swine 24 hours after dosing with RDX	C-9
Table C-3. Elimination $t_{1/2}$ values for RDX or radiolabeled RDX.....	C-10
Table C-4. Parameter values used in the Sweeney et al. (2012a) and Sweeney et al. (2012b) PBPK models for RDX in rats, humans, and mice as reported by authors	C-12
Table C-5. Parameters values used in the EPA application of the rat, human, and mouse models.....	C-15
Table C-6. Doses, dosing formulations, and absorption rate constants in animal and human studies	C-20
Table C-7. Sensitivity coefficients for rat and human RDX PBPK models	C-26
Table C-8. Summary of case reports of exposure to RDX	C-32
Table C-9. Occupational epidemiologic studies of RDX: summary of methodologic features ..	C-36
Table C-10. Evidence pertaining to mortality in animals	C-39
Table C-11. Evidence pertaining to other noncancer effects (hematological) in humans.....	C-47
Table C-12. Evidence pertaining to other noncancer effects in animals	C-49
Table C-13. Summary of in vitro studies of the genotoxicity of RDX.....	C-64
Table C-14. Summary of in vivo studies of the genotoxicity of RDX.....	C-68
Table C-15. Summary of in vitro and in vivo studies of the genotoxicity of RDX metabolites ..	C-69
Table D-1. Noncancer endpoints selected for dose-response modeling for RDX.....	D-1
Table D-2. Convulsion or mortality endpoints from Crouse et al. (2006) selected for dose-response modeling for RDX.....	D-2
Table D-3. Model predictions for convulsions in female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR= 1% ER.....	D-4
Table D-4. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER.....	D-6
Table D-5. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER.....	D-9
Table D-6. Model predictions for convulsions in female F344 rats exposed to RDX by gavage on GDs 6–19 (Cholakakis et al., 1980); BMR = 1% ER	D-11
Table D-7. Model predictions for combined incidence of convulsion and mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER	D-13
Table D-8. Model predictions for testicular degeneration in male B6C3F ₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% ER.....	D-15
Table D-9. Model predictions for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 10% ER.....	D-18
Table D-10. Mortality data selected for dose-response modeling for RDX	D-20
Table D-11. BMD modeling results for combined mortality in male and female F344 rats exposed to RDX by diet for 13 weeks (Levine et al., 1981b); BMR = 1% ER.....	D-23

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Table D-12. BMD modeling results for mortality (number found dead) in rats exposed to RDX in the diet for 13 weeks (von Oettingen et al., 1949) D-25

Table D-13. BMD modeling results for combined mortality (number found dead) in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 1% ER D-26

Table D-14. Model predictions for mortality in female Sprague-Dawley rats exposed to RDX by gavage on gestation days 6–15 (Angerhofer et al., 1986); BMR = 1% ER D-28

Table D-15. Cancer endpoints selected for dose-response modeling for RDX..... D-30

Table D-16. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 10% ER D-32

Table D-17. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984); BMR = 5% ER D-34

Table D-18. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 10% ER D-36

Table D-19. Model predictions for B6C3F₁ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 5% ER D-38

Table D-20. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months, using incidence frequencies from Parker et al. (2006) and sample sizes from Lish et al. (1984); BMR = 10% ER .. D-42

Table D-21. Liver carcinoma data from Levine et al. (1983)..... D-45

Table D-22. Model predictions and oral slope factor for alveolar/bronchiolar carcinomas in male B6C3F₁ mice exposed to RDX by diet for 2 years (Lish et al., 1984)..... D-46

Table D-23. Summary of BMD modeling results for model predictions for alveolar/bronchiolar carcinoma in male B6C3F₁ mice exposed to RDX by diet for 2 years (Lish et al., 1984); BMR = 10% ER D-46

Table D-24. Model predictions and oral slope factor for hepatocellular carcinomas in male F344 rats administered RDX in the diet for 2 years (Levine et al., 1983) D-49

Table D-25. Model predictions for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983); BMR = 5% ER ... D-50

FIGURES

Figure C-1. PBPK model structure for RDX in rats and humans.....	C-11
Figure C-2. EPA rat PBPK model predictions fitted to observed RDX blood concentrations in male and female Sprague-Dawley rats following i.v. exposure.....	C-17
Figure C-3. EPA rat PBPK model predictions fitted to observed RDX blood concentrations following oral exposure to RDX dissolved in water.....	C-18
Figure C-4. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to RDX in dry capsules.....	C-18
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. ..	C-19
Figure C-6. EPA rat model predictions fitted to observed RDX brain tissue concentrations following oral exposure to RDX.....	C-19
Figure C-7. EPA rat model predictions fitted to observed RDX blood concentrations following oral exposure to fine-grain RDX in a saline slurry.	C-21
Figure C-8. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic gavage study.....	C-22
Figure C-9. Comparison of EPA rat model predictions with data from Schneider et al. (1978) for the subchronic drinking water study.....	C-22
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).....	C-24
Figure C-11. EPA human model predictions fitted to observed RDX blood concentrations resulting from accidental exposure to adults assumed to be 70 kg (Özhan et al., 2003).....	C-24
Figure C-12. Comparison of EPA mouse PBPK model predictions with data from oral exposure to RDX dissolved in water.	C-27
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). ..	D-5
Figure D-2. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).	D-7
Figure D-3. Plot of incidence rate by dose, with the fitted curve of the multistage 3° model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% ER; dose shown in mg/kg-day.....	D-10
Figure D-4. Plot of incidence rate by dose, with the fitted curve of the selected model, for convulsions in female F344 rats exposed to RDX by gavage on GDs 6–19 (Cholakakis et al., 1980).....	D-12
Figure D-5. Plot of incidence rate by dose with fitted curve for Multistage 3° model for model predictions for combined incidence of convulsion and mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).	D-14
Figure D-6. Plot of incidence rate by dose, with fitted curve for selected model, for testicular degeneration in male B6C3F ₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984).....	D-16
Figure D-7. Plot of incidence rate by dose, with fitted curve for selected model, for prostate suppurative inflammation in male F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983).	D-19

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Figure D-8. Plot of incidence rate by dose, with the fitted curve of the multistage 2° model, for combined mortality in male and female F344 rats exposed to RDX by diet for 13 weeks (Levine et al., 1981b); BMR = 1% ER. D-24

Figure D-9. Plot of incidence rate by dose with fitted curve for Dichotomous-Hill model for Model predictions for mortality (number found dead) in rats exposed to RDX in the diet for 13 weeks (von Oettingen et al., 1949); dose shown in mg/kg-day. D-26

Figure D-10. Plot of incidence rate by dose, with the fitted curve of the multistage 2° model, for mortality in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006). BMR = 1% ER; dose shown in mg/kg-day..... D-27

Figure D-11. Plot of incidence rate by dose, with the fitted curve of the multistage 3° model, for mortality in female Sprague-Dawley rats exposed to RDX by gavage on gestation days 6–15 (Angerhofer et al., 1986); BMR = 1% ER..... D-29

Figure D-12. Plot of incidence rate by dose, with the fitted curve for the selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984). D-32

Figure D-13. Plot of incidence rate by dose, with fitted curve for selected model, for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984). D-34

Figure D-14. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Parker et al., 2006). D-36

Figure D-15. Plot of incidence rate by dose, with fitted curve for selected model, for B6C3F₁ female mouse combined hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for 24 months (Parker et al., 2006). D-38

Figure D-16. Plot of incidence rate by dose with fitted curve for Multistage-Cancer 1° model for model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months, using incidence frequencies from Parker et al. (2006) and sample sizes from Lish et al. (1984). D-42

Figure D-17. Plot of incidence rate by dose with fitted curve for Multistage-Cancer 1° model for Model predictions for alveolar/bronchiolar carcinoma in male B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984). D-47

Figure D-18. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in F344 rats exposed to RDX by diet for 24 months (Levine et al., 1983). D-50

ABBREVIATIONS

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

This document is a draft for review purposes only and does not constitute Agency policy.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

APPENDIX A. ASSESSMENTS BY OTHER NATIONAL AND INTERNATIONAL HEALTH AGENCIES

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

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

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

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

15 **B.1. DEFENSE TECHNICAL INFORMATION CENTER (DTIC) LITERATURE** 16 **SEARCH AND SCREEN**

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

6 The 85 unique selected citations with unlimited distribution were uploaded to the Health
7 and Environmental Research Online (HERO) website¹ (<http://hero.epa.gov>). The 10 citations with
8 limited distribution were subject to a more in-depth screen to determine whether the references
9 provided additional primary health effects data and whether the U.S. Environmental Protection
10 Agency (EPA) should seek authorization for public distribution and upload to HERO. A review of
11 the abstract or full-text of the documents associated with the limited-distribution citations resulted
12 in the following determinations:

- 13 • one citation was excluded because it did not provide additional primary health effects data.
14 The citation reported data from a study that was subsequently published ([Hathaway and](#)
15 [Buck, 1977](#)) and had already been identified by the literature search strategy.
- 16 • one citation (dated 1944) provided human and animal inhalation data and was considered
17 pertinent, but was not brought forward for further review because flaws in the design of
18 both the human and animal studies were such that results would not be considered
19 credible. Experimental animal study design issues included lack of a control group, small
20 numbers of animals, incomplete information on dosage or exposure levels, and inadequate
21 reporting. The human study described a case series and lacked a referent group and
22 measures of RDX exposure.
- 23 • eight citations were regulatory documents, reviews, or risk assessments that did not
24 specifically identify RDX and did not appear to contain primary health effects data.

25 Based on these determinations, none of the 10 limited distribution citations that were
26 subject to further review were selected for further consideration or added to HERO.
27

¹HERO is a database of scientific studies and other references used to develop EPA's assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 1.6 million scientific references, including articles from the peer-reviewed literature. New studies are added continuously to HERO.

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

This document is a draft for review purposes only and does not constitute Agency policy.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Database	Terms	Hits
Toxcenter Date: 4/2012	<p>((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 genotox? 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))</p> <p>Notes: Duplicates were removed; Biosis subfile results were date limited to avoid extensive overlap with Toxline.</p>	337 titles screened (20 selected for full records and added to HERO)
Toxcenter Date limit: 1/2012– 2/2013	<p>((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</p>	26 titles screened (6 selected for full records and added to HERO)

This document is a draft for review purposes only and does not constitute Agency policy.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Database	Terms	Hits
	<p>trinitramine" OR Trimethyleentritramine 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))</p> <p>Notes: Duplicates were removed.</p>	
<p>Toxcenter Date limit: 11/2012– 1/2014</p>	<p>((((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 Cyclotrimethylenetriamine OR Trimethylenetriamine OR "Trimethylene trinitramine" OR Trimethyleentritramine 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</p>	<p>20 titles screened (0 selected for full records; none added to HERO)</p>

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Database	Terms	Hits
	<p>(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))</p> <p>Notes: Duplicates were removed.</p>	
<p>Toxcenter Date limit: 11/2013– 1/2015</p>	<p>((((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 Trimethyleentritramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py >2013 OR ed>20131101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermator? OR spermatox? OR spermatoz? OR spermatu? OR spermi? OR spermo? OR neonat? OR newborn OR development OR developmental? OR zygote? OR child OR children OR adolescen? OR infant OR wean? OR offspring OR age(w)factor? OR dermal? OR dermis OR skin OR epiderm? OR cutaneous?</p>	<p>80 titles screened (3 selected for full records and added to HERO)</p>

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Database	Terms	Hits
	<p>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))</p> <p>Note: Duplicates were removed.</p>	
<p>Toxcenter</p> <p>Date limit: 11/2014– 5/2016</p>	<p>((((121-82-4 OR Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108") NOT (patent/dt OR tscats/fs)) AND (py >2013 OR ed>20131101)) AND (chronic OR immunotox? OR neurotox? OR toxicokin? OR biomarker? OR neurolog? OR pharmacokin? OR subchronic OR pbpk OR epidemiology/st,ct, it) OR acute OR subacute OR ld50# OR lc50# OR (toxicity OR adverse OR poisoning)/st,ct,it OR inhal? OR pulmon? OR nasal? OR lung? OR respir? OR occupation? OR workplace? OR worker? OR oral OR orally OR ingest? OR gavage? OR diet OR diets OR dietary OR drinking(w)water OR (maximum and concentration? and (allowable OR permissible)) OR (abort? OR abnormalit? OR embryo? OR cleft? OR fetus? OR foetus? OR fetal? OR foetal? OR fertil? OR malform? OR ovum OR ova OR ovary OR placenta? OR pregnan? OR prenatal OR perinatal? OR postnatal? OR reproduc? OR steril? OR teratogen? OR sperm OR spermac? OR spermag? OR spermati? OR spermas? OR spermatob? OR spermatoc? OR spermatog? OR spermatoi? OR spermatol? OR spermatior? 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))</p>	<p>33 titles screened (0 selected for full records and added to HERO)</p>

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Database	Terms	Hits
	Note: Duplicates were removed.	

1

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

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

This document is a draft for review purposes only and does not constitute Agency policy.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1

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

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

This document is a draft for review purposes only and does not constitute Agency policy.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1
 2 ^a[Sweeney et al. \(2012a\)](#) and [Sweeney et al. \(2012b\)](#) were selected for forward and backward searching in the Web
 3 of Science as the two more recent reviews of the health effects of RDX toxicity in the published literature.

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

C.1. TOXICOKINETICS

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is absorbed following exposure by inhalation and oral routes. The rate and extent of absorption are dependent upon the dosing preparation. RDX is systemically distributed, can be transferred from mother to fetus, and can transfer in maternal milk. Metabolism of RDX is extensive and includes denitration, ring cleavage, and generation of CO₂ possibly through cytochrome P450 (CYP450). RDX metabolites are eliminated primarily via urinary excretion and exhalation of CO₂.

C.1.1. Absorption

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

Oral Absorption

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

1 [al., 1986](#)). RDX was measured in serum, urine, cerebrospinal fluid, and feces. Based on a kinetic
2 analysis of the serum RDX concentrations, the dose was estimated to be 85 mg/kg and the first-
3 order absorption rate constants were estimated to be 0.34–2.20 hour⁻¹ ([Woody et al., 1986](#))².
4 [Sweeney et al. \(2012a\)](#) estimated the absorption rate constant for this same subject to be
5 0.060 hour⁻¹. The large range in the calculated absorption rate constants resulted from uncertainty
6 in the dose and time to peak serum RDX levels, and the models that were used to simulate the RDX
7 toxicokinetics. [Özhan et al. \(2003\)](#) summarized plasma RDX levels in five military personnel who
8 were accidentally exposed to toxic levels of RDX. Although [Özhan et al. \(2003\)](#) reported that
9 personnel were exposed through dermal and inhaled routes, secondary oral exposure may have
10 occurred. Based on physiologically based pharmacokinetic (PBPK) model fits to the plasma RDX
11 concentration data, [Sweeney et al. \(2012a\)](#) estimated a first-order absorption rate constant of
12 0.033 hour⁻¹. [Küçükardali et al. \(2003\)](#) summarized plasma RDX levels in five military personnel
13 who ingested toxic levels of RDX (doses were not reported). RDX was detected in plasma of all
14 patients within 3 hours after ingestion.

15 Quantitative data to directly support estimates of oral bioavailability are available from
16 studies in rats and mice ([Guo et al., 1985](#); [Schneider et al., 1978, 1977](#)). Results of single and
17 repeated oral dose studies in adult Sprague-Dawley rats indicate that approximately 83–87% of the
18 administered dose is absorbed from the gastrointestinal (GI) tract. Following gavage
19 administration of 50 mg/kg [¹⁴C]-RDX dissolved in dimethylsulfoxide (DMSO), approximately 90%
20 of the administered carbon-14 was recovered 4 days after dosing, with ~3% in feces, 34% in urine,
21 43% in expired air, and 10% in the carcass ([Schneider et al., 1977](#)). It is unclear if the carcass
22 includes the GI tract, which may include unabsorbed RDX. Assuming that all of the carbon-14 in
23 feces represents unabsorbed RDX (rather than RDX that was absorbed and subsequently secreted
24 into the intestine), results of this study indicate that at least 87% of the administered dose was
25 absorbed from the GI tract. Similar results were observed following repeated daily oral exposure of
26 Sprague-Dawley rats to [¹⁴C]-RDX by gavage (in DMSO) or drinking water for 1 week. Based on
27 recovery of carbon-14 in urine and expired air and the carbon-14 retained in carcass,
28 approximately 83% (drinking water) to 85 % (gavage) of the administered dose was absorbed
29 ([Schneider et al., 1978](#)).

30 An estimate of oral bioavailability in rats can also be obtained from data on blood RDX
31 concentrations reported in [Krishnan et al. \(2009\)](#). Male Sprague-Dawley rats received a single
32 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.
33 Estimates of bioavailability were obtained based on the reported blood RDX concentrations,
34 terminal elimination rate constants (estimated for this review by fitting the serum RDX data with a
35 first-order exponential function, see Table C-5 in Section C.1.4, Excretion) and the blood area under
36 the curve (AUC) values (calculated for this review using the trapezoid rule extrapolated to infinite

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

1 time). Calculated dose-adjusted AUC values were 9.6 and 8.4 hours·kg/L for the i.v. studies and
2 4.7 and 6.0 hours·kg/L for the oral dosing studies. These AUC values correspond to estimated oral
3 bioavailability ranging from 50 to 70%. Recovery of administered radiolabel was incomplete
4 (~90% of the administered carbon-14) in the studies ([Schneider et al., 1978, 1977](#)); therefore, it is
5 possible that oral bioavailability is actually higher than 83–87%. [Guo et al. \(1985\)](#) reported data on
6 blood tritium kinetics in mice that received i.v. (0.055 mg RDX or ~2.5 mg/kg body weight) or oral
7 (50 mg/kg) doses of [³H]-RDX. Based on the reported blood tritium concentrations (% of dose/g)
8 and terminal t_{1/2} values for concentrations of tritium in blood (1.1 days for i.v. and 2.2 days for
9 oral), the corresponding AUCs of the blood concentration versus time curves were calculated
10 (calculated for this review using the trapezoid rule extrapolated to infinite time) to be 30 and
11 16 hours·% dose/g for i.v. and oral dosing, respectively. This corresponds to an oral bioavailability
12 of RDX-derived tritium concentration of approximately 50% (i.e., 16/30).

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

19 Oral bioavailability of RDX appears to vary depending upon the physical form of RDX and
20 the matrix (e.g., soil, vegetation) in which it is administered. [Schneider et al. \(1977\)](#) compared the
21 oral absorption of a single 100 mg/kg gavage dose of coarse granular [¹⁴C]-RDX as a slurry in
22 isotonic saline with a single 50 mg/kg gavage dose of a finely powdered [¹⁴C]-RDX solution in saline
23 in Sprague-Dawley rats. Plasma carbon-14 levels were measured for 24 hours after dosing. For
24 both [¹⁴C]-RDX preparations, peak plasma levels of carbon-14 were observed 24 hours after oral
25 administration, with a higher 24-hour plasma concentration for the 50 mg/kg dose (~4.7 µg/mL)
26 compared to the 100 mg/kg dose (3.12 µg/mL). Results of this study indicate that the oral
27 bioavailability of RDX may be greater for the finely powdered preparation than for the coarse
28 granular preparation consistent with a greater surface area available for absorption with finely
29 powdered RDX. However, blood levels were only measured 24 hours after dosing, and the lower
30 24-hour carbon-14 plasma concentration for the coarse granular preparation could be due to
31 slower absorption of coarse RDX granules compared with fine RDX powder, rather than lower
32 overall bioavailability.

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

1 Plant (AAP) or Fort Meade. Blood concentrations for rats dosed with Louisiana AAP soil
2 (1.24 mg/kg) and neat RDX (1.24 mg/kg) peaked at approximately 6 hours. The AUC and 6-hour
3 RDX blood concentration were both approximately 25% lower for Louisiana AAP soil than for neat
4 RDX ($p \leq 0.003$ for AUC), suggesting that oral bioavailability of RDX from Louisiana AAP soil was
5 25% lower than neat RDX. For Fort Meade soil (0.2 mg/kg), RDX blood concentrations peaked at
6 6 hours compared to 4 hours for neat RDX (0.2 mg/kg). The 4-hour blood concentration for Fort
7 Meade soil was approximately 15% lower than for neat RDX, although the AUC for Fort Meade soil
8 was only 5% lower than for neat RDX (not statistically significant). Collectively, these results
9 suggest that RDX in soil is absorbed following oral exposure and that it has a lower bioavailability
10 than neat RDX.

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

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

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

1 single dose (100 mg/kg) of RDX administered as a finely powdered in saline ([Bannon et al., 2009a](#);
2 [Schneider et al., 1977](#)). Peak plasma concentrations in Yucatan miniature swine administered a
3 single dose of [¹⁴C]-RDX (45 mg/kg as a suspension in carboxymethylcellulose) were reached
4 within 6–12 hours after dosing ([Musick et al., 2010](#)). [Krishnan et al. \(2009\)](#) and [Sweeney et al.](#)
5 [\(2012a\)](#) estimated absorption rates in rats dosed with higher doses of coarse granular RDX to be
6 approximately 100 times slower than absorption rates in rats dosed with lower doses of finely
7 powdered neat RDX preparations or neat RDX dissolved in aqueous vehicles. For example,
8 [Krishnan et al. \(2009\)](#) estimated the absorption rate constant to be 0.75 hour⁻¹ for rats dosed with
9 neat RDX dissolved in water (1.53 or 2.07 mg/kg) or neat RDX in a gelatin capsule (0.2 or
10 1.24 mg/kg) ([Crouse et al., 2008](#)), compared to 0.0075 hour⁻¹ for rats dosed with coarse granular
11 RDX (100 mg/kg) ([Schneider et al., 1977](#)).

12 ***Inhalation Absorption***

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

22 ***Dermal Absorption***

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

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

1 **C.1.2. Distribution**

2 Information on the distribution of absorbed RDX in humans is limited to a few cases of
3 accidental exposures to RDX that provide data on the kinetics of RDX in blood and cerebrospinal
4 fluid ([Küçükardali et al., 2003](#); [Özhan et al., 2003](#); [Woody et al., 1986](#)). Concentrations of RDX in
5 serum and cerebrospinal fluid were similar (11 and 9 mg/L, respectively) in a child 24 hours after
6 ingesting an estimated dose of 85 mg/kg RDX ([Woody et al., 1986](#)). More extensive information on
7 tissue distribution is available for animals, including mice, rats, and swine ([Musick et al., 2010](#);
8 [Bannon, 2006](#); [Reddy et al., 1989](#); [Guo et al., 1985](#); [MacPhail et al., 1985](#); [Schneider et al., 1977](#)). In
9 these studies, RDX or radiolabeled RDX ($[^{14}\text{C}]$ or $[^3\text{H}]$) was administered by the oral, intraperitoneal
10 (i.p.), i.v., or intratracheal route and the distribution of the RDX or radiolabel was measured. Since
11 metabolism of RDX can result in loss of carbon-14 or tritium from the parent compound, the
12 distribution of radiolabel will not necessarily reflect the distribution of RDX ([Schneider et al., 1977](#)).
13 To compare tissue distributions in studies in which animals received different doses by different
14 routes of administration, distribution data are expressed as ratios of tissue RDX or radiolabel to
15 that of either whole blood or plasma, whichever was reported. RDX in blood distributes into red
16 blood cells (RBCs) and plasma to achieve concentration ratios that are close to unity. The
17 plasma:whole blood carbon-14 ratio in swine that received a single oral dose of $[^{14}\text{C}]$ -RDX
18 (45 mg/kg) was approximately 1.3 ([Musick et al., 2010](#)), and whole rat blood incubated in vitro
19 with RDX had a plasma:RBC RDX ratio of approximately 1.0 ([Krishnan et al., 2009](#)). As a result of
20 the similarity between plasma and whole blood concentrations, tissue distribution is approximately
21 equivalent when expressed as ratios of blood or plasma.

22 Studies conducted in rats, mice, and swine indicate that absorbed RDX distributes to many
23 different tissues. [Schneider et al. \(1977\)](#) estimated the volume of distribution of RDX to be
24 approximately 2.18 L/kg in rats, based on plasma RDX kinetics in rats that received a single i.p.
25 dose of RDX (5–6 mg/kg). Consistent with this estimate are observations of tissue:blood (or
26 plasma) concentration ratios that exceed 1 in various tissues, including brain (showing that RDX
27 can cross the blood:brain barrier), heart, kidney, and liver ([Musick et al., 2010](#); [Bannon et al., 2006](#);
28 [MacPhail et al., 1985](#); [Schneider et al., 1977](#)). Distribution within the brain may not be uniform.
29 However, [Bannon et al. \(2006\)](#) observed tissue:blood concentrations for RDX of approximately 4 in
30 brain hippocampus and 3.5 in brain cortex of swine that received a single oral dose of 10 mg/kg
31 $[^{14}\text{C}]$ -RDX, although this is the only study that reported distribution for brain regions. Reported
32 tissue:blood (or plasma) concentration ratios of RDX 24 hours following a single dose (oral or i.p.)
33 were 1–9 for kidney, 1–7 for liver, and 1–3 for heart (Table C-1) ([Bannon, 2006](#); [Schneider et al.,](#)
34 [1977](#)). With repeated oral dosing (e.g., 30–90 days), tissue:blood ratios of RDX for these tissues are
35 consistently greater than unity ([Schneider et al., 1978](#)). There is no consistent evidence that RDX
36 accumulates in fat, although estimates of the fat:blood partition coefficient range from 6 to 8 and
37 exceed that of other tissues ([Sweeney et al., 2012a](#); [Krishnan et al., 2009](#)).

1 **Table C-1. Distribution of RDX or radiolabel from administered RDX^a**

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

2
3 ^aValues are tissue:blood or tissue:plasma ratios following a single dose of either RDX, [¹⁴C]-RDX, or [³H]-RDX.

4 ^bCarbon-14

5 ^cTissue:plasma

6 ^dRDX

7 ^eTritium

8 ^fTissue:blood

9 ^gNot available

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

17 C.1.3. Metabolism

18 The metabolism of RDX is not well characterized. No studies investigating the metabolism
19 of RDX in humans were identified. Studies in animals indicate that RDX undergoes extensive
20 metabolism, including denitration, ring cleavage, and generation of CO₂. Predominant metabolic
21 pathways and major organs involved in RDX metabolism have not been identified, although results
22 of in vitro studies suggest a role for CYP450.

23 RDX undergoes metabolism through processes that generate CO₂. In Sprague-Dawley rats
24 administered a single 50 mg/kg gavage dose of [¹⁴C]-RDX, 43% was recovered as exhaled [¹⁴CO₂]
25 after 4 days ([Schneider et al., 1977](#)). Similarly, approximately 30–50% of the radioactivity was
26 recovered as exhaled [¹⁴CO₂] in rats administered [¹⁴C]-RDX in saturated drinking water or daily
27 gavage for up to 3 months ([Schneider et al., 1978](#)). Metabolism of RDX to CO₂ was also observed in
28 prairie voles following dietary exposure (average RDX dose per animal of 2.3 mg/kg-day) to

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

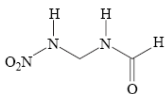
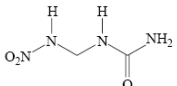
1 [14C]-RDX incorporated plant materials for 5–7 days, with approximately 9% of the administered
2 [14C]-RDX dose eliminated as exhaled [14CO₂] ([Fellows et al., 2006](#)).

3 Terminal metabolites of RDX have been identified in the urine of rats and swine, with very
4 little urinary excretion of parent compound, indicating extensive metabolism of RDX. Following
5 oral administration of a single 50 mg/kg gavage dose of [14C]-RDX, 3.6% of the urinary radioactivity
6 was identified as unmetabolized RDX ([Schneider et al., 1977](#)). Total urinary radiolabel accounted
7 for about one-third of the administered label and unmetabolized RDX contributed 3–5% of total
8 urinary radioactivity in rats exposed to [14C]-RDX-saturated drinking water for 1 or 13 weeks
9 ([Schneider et al., 1978](#)). Similar results were observed in Yucatan swine administered a single
10 45 mg/kg oral dose of [14C]-RDX, with approximately 1–3.5% of the urinary radioactivity as parent
11 RDX ([Major et al., 2007](#)). Urinary metabolites were not characterized in these studies ([Schneider et
12 al., 1978, 1977](#)). However, [Schneider et al. \(1978\)](#) cited unpublished findings in their laboratory
13 that, in addition to carbon dioxide, other one-carbon intermediates were produced, including
14 bicarbonate and formic acid.

15 In the environment, the predominant breakdown products of RDX are methylene
16 dinitramine and 4-nitro-2-diazbutanal ([Sweeney et al., 2012b](#); [Paquet et al., 2011](#)). RDX
17 metabolism in animals is less well understood. N-Nitroso RDX metabolites have been identified as
18 derived through anaerobic metabolism ([ATSDR, 2012](#); [Pan et al., 2007b](#)). Based on characterization
19 of RDX metabolites in urine and plasma of miniature swine, metabolism of RDX appears to involve
20 loss of nitro groups and ring cleavage ([Musick et al., 2010](#); [Major et al., 2007](#)). The two principal
21 urinary metabolites identified in miniature swine following a single oral dose of 43 or 45 mg/kg
22 were 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diaza-butanamide (see Table C-2). [Bhushan et al.
23 \(2003\)](#) suggested that the formation of the 4-nitro-2,4-diazabutanal metabolite occurred via
24 denitration followed by hydroxylation and spontaneous hydrolytic decomposition resulting in ring
25 cleavage and aldehyde formation. In the miniature swine gavage studies, only trace amounts of the
26 nitrosamine RDX metabolites, hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and 1-nitro-
27 3,5-dinitroso-1,3,5-triazacyclohexane (DNX), were found in urine ([Musick et al., 2010](#); [Major et al.,
28 2007](#)). In plasma, most of the radioactivity existed as RDX, with trace levels of MNX, DNX, and
29 1,3,5-trinitroso-1,3,5-triazacyclohexane (TNX). The study authors suggested that the trace levels of
30 MNX, DNX, and TNX in plasma may have been formed within the GI tract via sequential nitrogen
31 reduction by intestinal bacteria ([Major et al., 2007](#)). The low levels of these compounds in urine
32 and plasma were attributed to the nearly complete absorption of RDX from the GI tract, leaving
33 little parent compound available for bacterial metabolism within the GI tract. In a study of female
34 deer mice (*Peromyscus maniculatus*) fed diets containing RDX at concentrations of 12 and
35 120 mg/kg for 9 days, MNX and DNX were identified in the stomach, but TNX was not detected ([Pan
36 et al., 2007b](#)). MNX and DNX were also measured in various organs of female B6C3F₁ mice provided
37 RDX in feed at doses of 0.38–522 mg/kg; TNX was also detected in some organ compartments, but
38 not in the liver. The authors concluded that RDX can be metabolized into its N-nitroso compounds

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

5 **Table C-2. Principal urinary metabolites of RDX in miniature swine 24 hours**
 6 **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	

7
 8 Sources: [Major et al. \(2007\)](#); [Musick et al. \(2010\)](#)
 9

10 Although the metabolic pathways and major tissues involved in RDX metabolism have not
 11 been identified, there is some evidence for the involvement of the liver and CYP450 enzymes.
 12 Comparison of hepatic radioactivity to liver concentrations of RDX after a single gavage dose to rats
 13 suggested the presence of RDX metabolites and a possible role for hepatic metabolism of RDX
 14 ([Schneider et al., 1977](#)). In vitro data indicated that CYP450 may be involved in the metabolism of
 15 RDX ([Bhushan et al., 2003](#)). Incubation of RDX with nicotinamide adenine dinucleotide phosphate
 16 (NADPH) and rabbit liver CYP450 2B4 under anaerobic conditions produced nitrite, 4-nitro-
 17 2,4-diazabutanal, formaldehyde, and ammonium ion ([Bhushan et al., 2003](#)). The reaction rate
 18 under aerobic conditions was approximately one-third of that observed under anaerobic
 19 conditions. Several CYP450 inhibitors (ellipticine, metyrapone, phenylhydrazine, 1-aminobenzo-
 20 triazole, and carbon monoxide) decreased the formation of RDX metabolites (55–82% inhibition),
 21 providing support for the role of CYP450 in RDX metabolism.

22 **C.1.4. Excretion**

23 The primary routes of elimination of absorbed RDX are excretion of RDX and metabolites in
 24 urine, and exhalation of CO₂ liberated from metabolism of RDX ([Sweeney et al., 2012a](#); [Musick et al.,](#)
 25 [2010](#); [Krishnan et al., 2009](#); [Major et al., 2007](#); [Schneider et al., 1977](#)). Tritium derived from
 26 administered [³H]-RDX has been detected in mouse gall bladder contents, suggesting biliary
 27 secretion in this species ([Guo et al., 1985](#)); however, biliary secretion of RDX or metabolites has not
 28 been confirmed in other animal species. Studies conducted in the rat and swine suggest that
 29 metabolism is the dominant mechanism of elimination of absorbed RDX. In both species,
 30 metabolites dominated the carbon-14 distribution in urine of animals that received doses of

1 [14C]-RDX, with RDX accounting for <5% of the urinary carbon-14 ([Musick et al., 2010](#); [Schneider et](#)
2 [al., 1977](#)).

3 Data on kinetics of elimination of absorbed RDX from blood are available from reports of
4 accidental exposures of humans to RDX (Table C-3). [Woody et al. \(1986\)](#) estimated the elimination
5 $t_{1/2}$ to be approximately 15 hours in a child who ingested approximately 85 mg of RDX per kg of
6 body weight. The $t_{1/2}$ estimate was based on measured serum concentrations of RDX made
7 between 24 and 120 hours following ingestion for RDX. Based on plasma RDX concentration data
8 from five adults exposed to RDX (measurements made between 24 and 96 hours following
9 exposure) ([Özhan et al., 2003](#)), a first-order elimination $t_{1/2}$ of 20–30 hours was derived (calculated
10 for this review by fitting the serum RDX data to a first-order exponential function). It needs to be
11 noted that it is not possible to draw reliable inferences from these values since they are based on
12 accidental, acute exposures and, in particular, the data for the child are based on a single set of
13 measurements for one individual.

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

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

15
16 ^aObservation period following exposure on which the $t_{1/2}$ values were based.

17 ^bReported estimate of dose based on blood kinetics.

18 ^cValue for blood RDX.

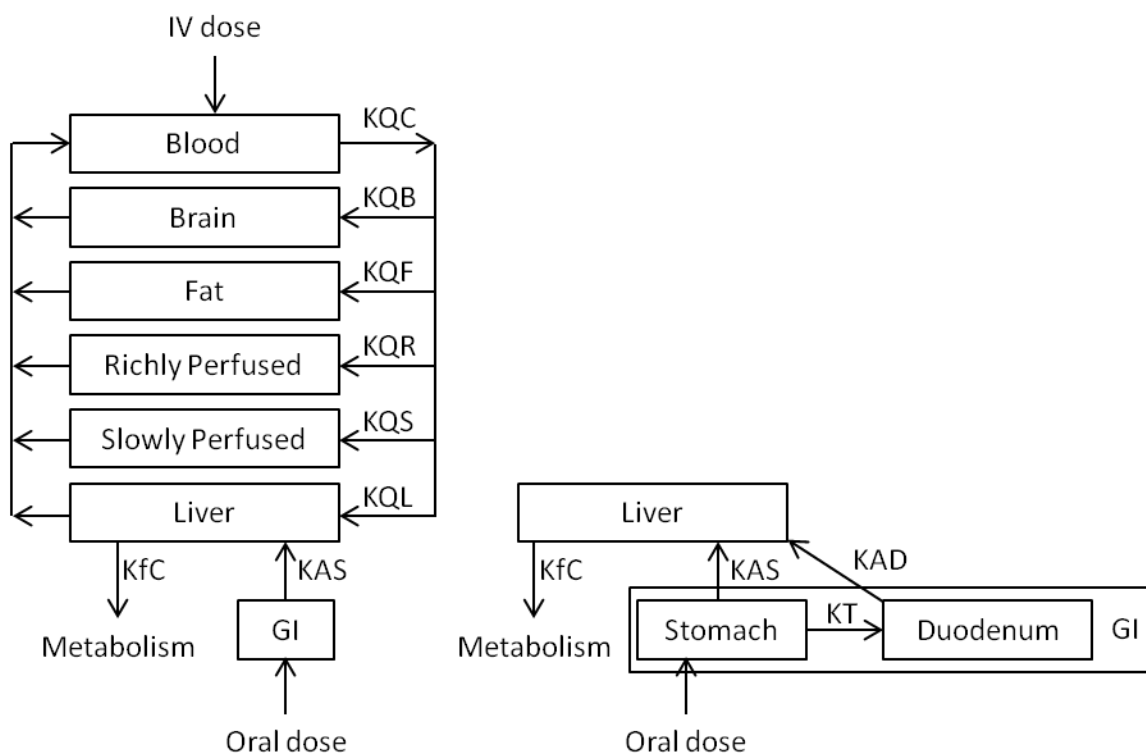
19 ^dCalculated for this review based on reported blood RDX concentrations.

20
21
22 The kinetics of elimination of absorbed RDX from blood has been evaluated in rats and
23 mice. In rats elimination kinetics were biphasic ([Krishnan et al., 2009](#); [Guo et al., 1985](#); [Schneider et](#)
24 [al., 1977](#)). As shown in Table C-3, estimated $t_{1/2}$ values for the terminal elimination phase in rats
25 range from 5 to 10 hours ([Krishnan et al., 2009](#); [Schneider et al., 1977](#)). Blood concentration time
26 course measurements of RDX can be used to estimate an apparent metabolism and elimination of
27 RDX from blood. The RDX blood concentrations reported in [Sweeney et al. \(2012b\)](#) after gavage
28 dosing of 35, 60, and 80 mg/kg RDX found a consistent terminal elimination $t_{1/2}$ of approximately
29 1.2 hours. The elimination $t_{1/2}$ estimated for rats ([Krishnan et al., 2009](#); [Schneider et al., 1977](#)) is as
30 much as an order of magnitude longer than mice ([Sweeney et al., 2012b](#)).

1 **C.1.5. Physiologically Based Pharmacokinetic (PBPK) Models**

2 **Overview of Available PBPK Models**

3 A PBPK model to simulate the pharmacokinetics of RDX in rats was first developed by
 4 [Krishnan et al. \(2009\)](#) and improved upon to extend the model to humans and mice ([Sweeney et al.](#)
 5 [2012a](#); [Sweeney et al., 2012b](#)). The [Sweeney et al. \(2012a\)](#) model consists of six main
 6 compartments: blood, brain, fat, liver, and lumped compartments for rapidly perfused tissues and
 7 slowly perfused tissues (Figure C-1). The model can simulate RDX exposures via the i.v. or oral
 8 route. Distribution of RDX to tissues is assumed to be flow-limited. Oral absorption is represented
 9 in this model as first-order uptake from the GI tract into the liver, with 100% of the dose absorbed.
 10 RDX is assumed to be cleared by first-order metabolism in the liver. However, there is no
 11 representation of the kinetics of any RDX metabolites. The acslX model code (Advanced Continuous
 12 Simulation Language, Aegis, Inc., Huntsville, Alabama) was obtained from the authors of [Sweeney et](#)
 13 [al. \(2012a\)](#).
 14



15
 16 Exposure to RDX is by the i.v. or oral route, and clearance occurs by metabolism in the liver. See Table C-4 for
 17 definitions of parameter abbreviations. The GI tract is represented as one compartment in [Krishnan et al. \(2009\)](#)
 18 (on the left) and two compartments in [Sweeney et al. \(2012a\)](#) (on the right).

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

20

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 The parameter values used in the [Sweeney et al. \(2012a\)](#) rat model are listed in Table C-4.
 2 The physiological model parameter values for cardiac output, tissue volumes, and blood perfusion
 3 of tissues were obtained from the literature ([Timchalk et al., 2002](#); [Brown et al., 1997](#)). RDX
 4 tissue:blood partition coefficients for liver (PL), brain (PB), and richly perfused tissues (PR) were
 5 estimated with an algorithm that relates the measured n-octanol:water partition coefficient for RDX
 6 to reported compositions of water and lipids in specific rat tissues ([Poulin and Theil, 2000](#); [Poulin
 7 and Krishnan, 1995](#)). Tissue:blood partition coefficients for fat (PF) and slowly perfused tissues
 8 (PS), as well as the metabolic rate constant (KfC), were simultaneously optimized to fit rat blood
 9 RDX concentrations following i.v. doses of 0.77 or 1.04 mg/kg RDX ([Krishnan et al., 2009](#))
 10 producing values of 5.57, 0.15, and 2.6 kg^{0.33}/hour for PF, PS, and KfC, respectively. While the
 11 optimized value for PS is much smaller than that used by [Krishnan et al. \(2009\)](#) (1.0 kg^{0.33}/hour),
 12 the optimized values for PF and KfC were fairly similar to those used by [Krishnan et al. \(2009\)](#)
 13 (7.55, and 2.2 kg^{0.33}/hour). The rat model with these parameter values also had good agreement
 14 with blood RDX concentrations after a 5–6 mg/kg i.v. exposure ([Schneider et al., 1977](#)).

15 **Table C-4. Parameter values used in the [Sweeney et al. \(2012a\)](#) and [Sweeney
 16 et al. \(2012b\)](#) PBPK models for RDX in rats, humans, and mice as reported by
 17 authors**

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

- 1
- 2 ^aPredicted from n-octanol:water partition coefficient.
- 3 ^bOptimized from rat i.v. data.
- 4 ^cOptimized from human data of [Özhan et al. \(2003\)](#) and [Woody et al. \(1986\)](#).
- 5 ^dOptimized from mouse oral data.
- 6 ^eOptimized from rat oral data of [Bannon et al. \(2009a\)](#), [Crouse et al. \(2008\)](#), [Krishnan et al. \(2009\)](#), and [Schneider et al. \(1977\)](#).
- 7
- 8

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

11

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

1 so KT was set equal to zero making the GI submodel equivalent to a one-compartment model. The
2 value of KAS was adjusted separately to fit the oral studies with RDX in capsules ([Bannon et al.,
3 2009a](#); [Crouse et al., 2008](#)) and coarse-grain RDX in a saline slurry ([Schneider et al., 1977](#)).

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

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

24 Mouse RDX toxicokinetics were also modeled by [Sweeney et al. \(2012b\)](#) using the same
25 model structure as for rats. Values for the mouse physiological parameters such as tissue volumes
26 and blood perfusion of tissues were assumed to be the same as the body weight normalized
27 parameter values in the rat model. RDX tissue:blood partition coefficients for liver (PL), brain (PB),
28 and richly perfused tissues (PR) were estimated with an algorithm that relates the measured
29 n-octanol:water partition coefficient for RDX to reported compositions of water and lipids in
30 specific mouse tissues ([Poulin and Theil, 2000](#); [Poulin and Krishnan, 1995](#)). The K_{fC} and KAS were
31 optimized to fit measured mouse RDX blood concentrations ([Sweeney et al., 2012b](#)). The K_{fC} value
32 estimated for the mouse (102 kg^{0.33}/hour) is much higher than those estimated for rats and humans
33 (2.6 and 11.2 kg^{0.33}/hour, respectively); however, the KAS value (0.51/hour) fit to mouse data is
34 similar to the value (0.83/hour) used in the RDX rat model for gavage in water. The [Sweeney et al.
35 \(2012b\)](#) model predictions of blood RDX concentrations were in good agreement with the
36 experimental mouse gavage data reported in the same study.

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

1 **PBPK Model Evaluation and Further Development of the [Sweeney et al. \(2012a\)](#) and [Sweeney et al. \(2012b\)](#) Models**

3 EPA evaluated and performed a quality control check of the PBPK models for RDX in rats,
4 humans, and mice published by Sweeney and colleagues ([Sweeney et al., 2012a](#); [Sweeney et al.,](#)
5 [2012b](#)). The conclusions from these analyses are summarized below and then discussed in more
6 detail:

- 7 1) The model code and the parameter values matched the published reports. Minor
8 discrepancies in physiological parameters (KVR and KQS) were identified and updated in
9 the model by EPA.
- 10 2) The absorption of RDX from the GI tract did not use a consistent structure; for gavage doses,
11 the model used a two-compartment GI submodel and for other oral exposures (e.g., gelatin
12 capsule), the model used a one-compartment GI submodel. The model was revised to have
13 a one-compartment GI submodel to simulate all oral exposures with a consistent set of
14 absorption parameters for each dosage formulation of administered RDX.
- 15 3) Additional oral rat data were identified from single-dose studies ([MacPhail et al., 1985](#);
16 [Schneider et al., 1977](#)) and subchronic studies ([Schneider et al., 1978](#)) and were used for
17 model calibration as well as for independent comparison against model predictions.
- 18 4) In addition to the sensitivity analysis conducted by [Sweeney et al. \(2012b\)](#) on the mouse
19 model, a sensitivity analysis in the rat and human models was performed.
- 20 5) The [Sweeney et al. \(2012b\)](#) mouse model used the same physiological parameters scaled to
21 body weight as the rat model. This mouse model was revised to use mouse-specific
22 physiological parameters.

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

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

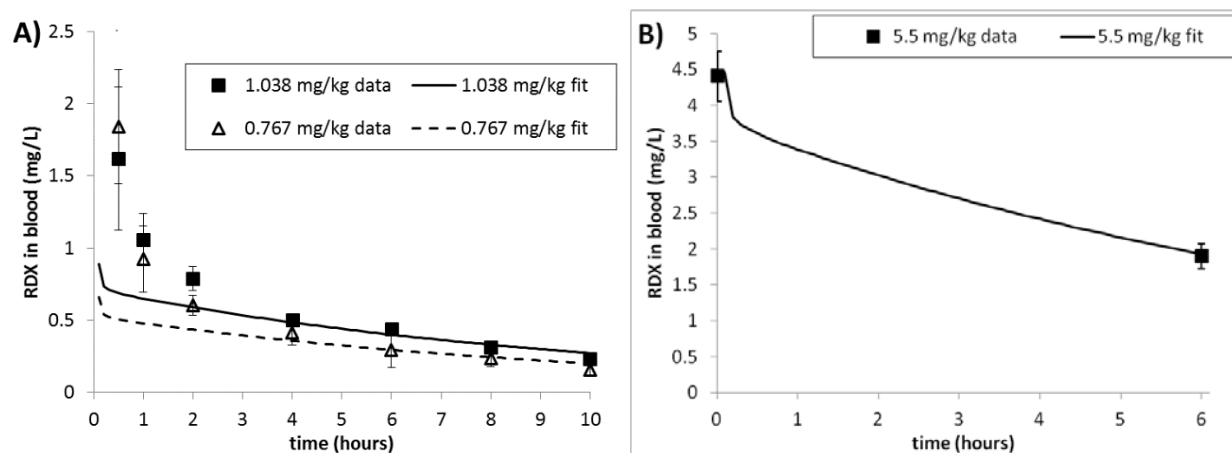
1
2
3
4
5
6

^aPredicted from n-octanol:water partition coefficient.

^bOptimized from rat i.v. data.

^cOptimized from human data of [Özhan et al. \(2003\)](#) and [Woody et al. \(1986\)](#).

^dOptimized from mouse oral data, and differs from that obtained by [Sweeney et al. \(2012b\)](#).



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

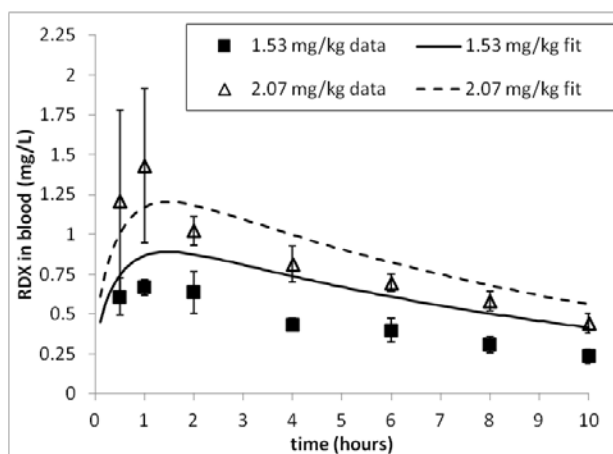
Figure C-2. EPA rat PBPK model predictions fitted to observed RDX blood concentrations in male and female Sprague-Dawley rats following i.v. exposure.

8 *Absorption of RDX from the GI Tract*

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

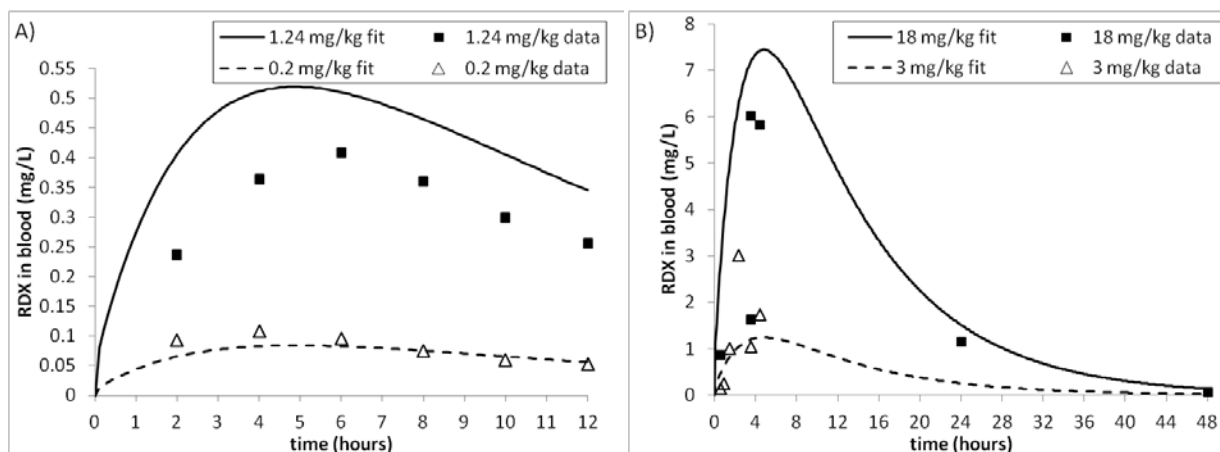
15 To follow the rule of model parsimony (i.e., use no more parameters than needed for the
 16 best fit to all of the data), oral absorption was modeled with a one-compartment GI tract submodel
 17 for all simulations. To account for the differences in absorption due to the physical form of RDX,
 18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

1 course data of these studies are similar to the fits of the [Sweeney et al. \(2012a\)](#) rat model. The fits
 2 to RDX brain time course data after oral exposure to RDX in capsules (Figure C-6A) are similar to
 3 the fits of the [Sweeney et al. \(2012a\)](#) rat model. The absorption rate constants for each dosing
 4 formulation are listed in Table C-6.
 5



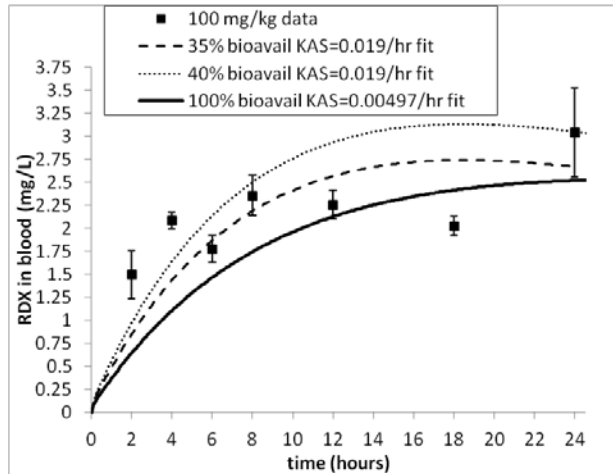
6
 7 Male and female Sprague-Dawley rats (0.4 kg) were dosed by gavage ([Krishnan et al., 2009](#)).

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



11 The ingested RDX doses were: A) 0.2 and 1.24 mg/kg RDX in male Sprague-Dawley rats (0.4 kg,
 12 data from [Crouse et al. \(2008\)](#)) and B) 3 and 18 mg/kg RDX in male and female Sprague-Dawley
 13 rats (0.35 kg, data from [Bannon et al. \(2009a\)](#)) for KAS = 0.35/hour.
 14

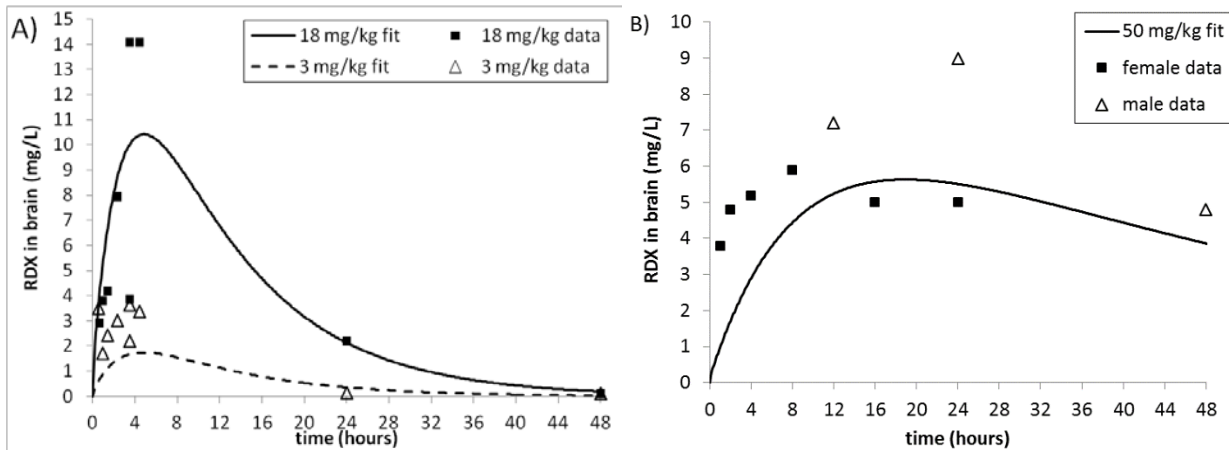
15 **Figure C-4. EPA rat model predictions fitted to observed RDX blood**
 16 **concentrations following oral exposure to RDX in dry capsules.**



1
2
3
4
5
6
7
8
9

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

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



13
14
15
16

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

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

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

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

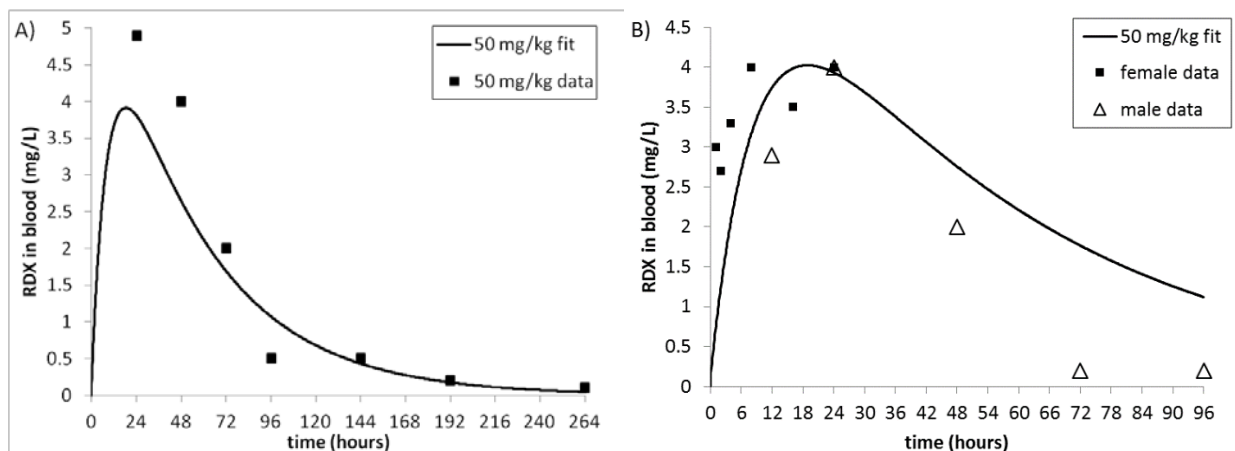
3
 4 ^aCapsules were filled with dry RDX from stock solution of acetone, and acetone was evaporated off.

5 ^bRDX particle size was ≤66 μm in diameter suspended in a 2% solution of carboxymethylcellulose.

6
 7 An alternative to varying the KAS for each RDX formulation would be to vary the oral
 8 bioavailability, in effect modifying the administered exposure concentration. Therefore, the
 9 sensitivity of the model fit to variations in oral bioavailability was examined in Figure C-5 and an
 10 analysis of model sensitivity to oral bioavailability was conducted as discussed further in the
 11 section, *Sensitivity Analysis of the Rat PBPK Model*.

12 ***Additional RDX Time-Course Data***

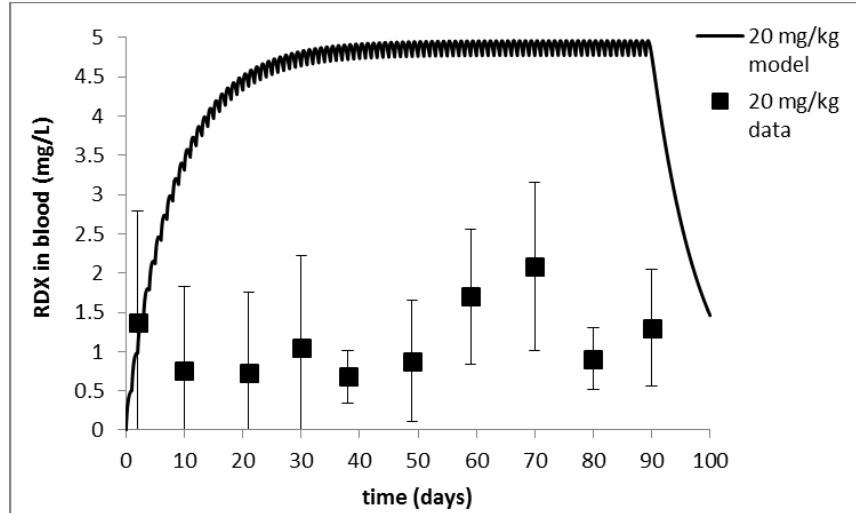
13 The EPA revised models were simultaneously fitted against additional RDX time-course
 14 data (not used in the original [Sweeney et al. \(2012a\)](#) model calibration). These data came from
 15 (1) two studies in which animals received oral doses of fine-grain RDX ([MacPhail et al., 1985](#);
 16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



1 Oral doses of 50 mg/kg RDX were administered to: A) male Sprague-Dawley rats (0.225 kg)
 2 ([Schneider et al., 1977](#)) and B) male and female Sprague-Dawley rats (0.25 kg) data ([MacPhail et](#)
 3 [al., 1985](#)). Best fit KAS = 0.019/hour.
 4

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

7
 8 Following calibration, the EPA model was further tested by comparison with results from
 9 two other subchronic oral studies in male and female rats ([Schneider et al., 1978](#)). These were a
 10 gavage study where 20 mg/kg RDX was administered in saline slurry and a drinking water study
 11 where rats were provided with RDX-saturated drinking water (50–70 µg/mL) ad libitum for which
 12 the study authors estimated a daily dose between 5 and 8 mg RDX/kg body weight. It is striking
 13 that the observed RDX blood concentrations in the gavage study (Figure C-8, symbols) were
 14 virtually the same, or only slightly elevated, as compared to the blood concentrations reported in
 15 the drinking water exposures, with an approximately threefold lower daily administered dose in
 16 the drinking water study (Figure C-9, symbols). This is counter to the expectation that higher doses
 17 cause higher blood levels and is discussed further below.



1

2

3

4

5

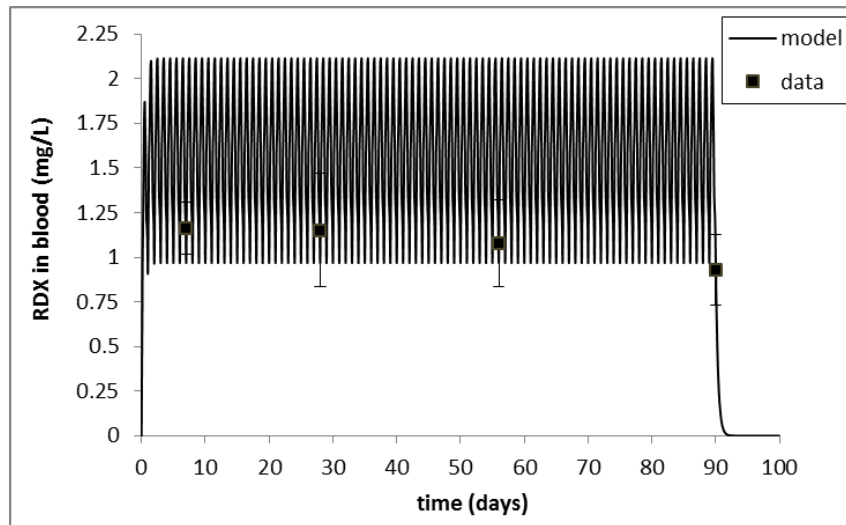
Model fits and mean observed RDX blood concentrations resulting from daily gavage doses of 20 mg/kg RDX for 90 days to male and female Sprague-Dawley rats (0.225 kg). The RDX in saline slurry was assumed to be coarse-grained with an oral absorption rate constant KAS = 0.00497/hour.

6

7

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

8



9

10

11

12

13

14

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

15

16

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

1 EPA's modified PBPK model was set up to simulate drinking water exposures with a
2 noncontinuous sipping pattern based on [Spiteri \(1982\)](#), which assumed 80% of the consumption to
3 occur episodically at night when the rats were awake³. The model predicts blood concentrations to
4 increase in proportion to the total dose; for the gavage study, the model predictions yielded an RDX
5 blood concentration approximately threefold higher than the reported mean blood concentrations
6 (Figure C-8), while for the subchronic drinking water study, the model fit the data reasonably well
7 (Figure C-9).

8 It is possible that multiple mechanisms such as elimination of unabsorbed RDX or metabolic
9 induction may explain why the observed RDX blood concentrations did not increase in proportion
10 to the higher administered dose in the gavage studies compared to the drinking water study.
11 Elimination of unmetabolized RDX may be an insignificant factor in the single-dose studies used for
12 calibration of the absorption constant for the RDX in saline slurry, but for repeated, higher doses
13 this elimination route could be significant. [Schneider et al. \(1978\)](#) found similar RDX
14 concentrations in the feces of rats in the gavage and drinking water studies (3.1 ± 2.0 and
15 2.7 ± 1.3 μg RDX per g dry weight feces, respectively). The total recovery of radioactivity in feces
16 was also similar in the gavage study ($4.8 \pm 0.8\%$, week 1 only) and drinking water study
17 ($4.4 \pm 0.6\%$, measured over the course of the study). Thus, the difference in fecal elimination for
18 the two routes does not appear significant.

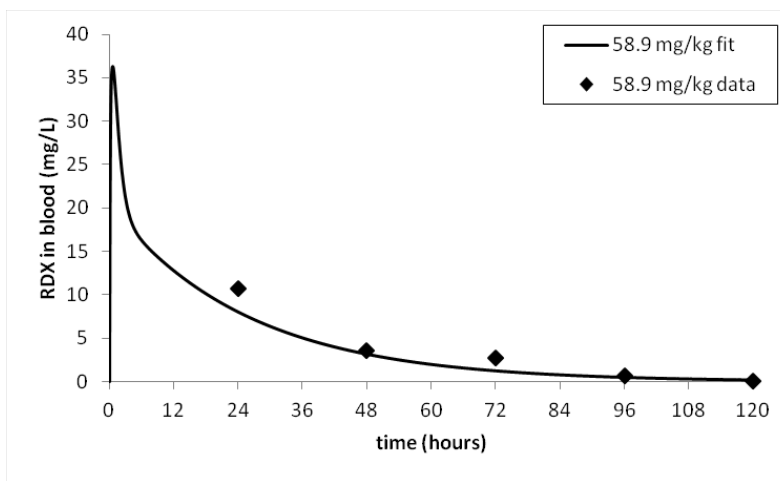
19 It is also possible that metabolic induction occurred during the repeated dosing of RDX in
20 the gavage study leading to the lower observed RDX blood concentrations. The reasonably good fits
21 of the model to the drinking water data set demonstrated achievement of regular periodic levels,
22 and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

27 ***Simulating Exposures in Humans***

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

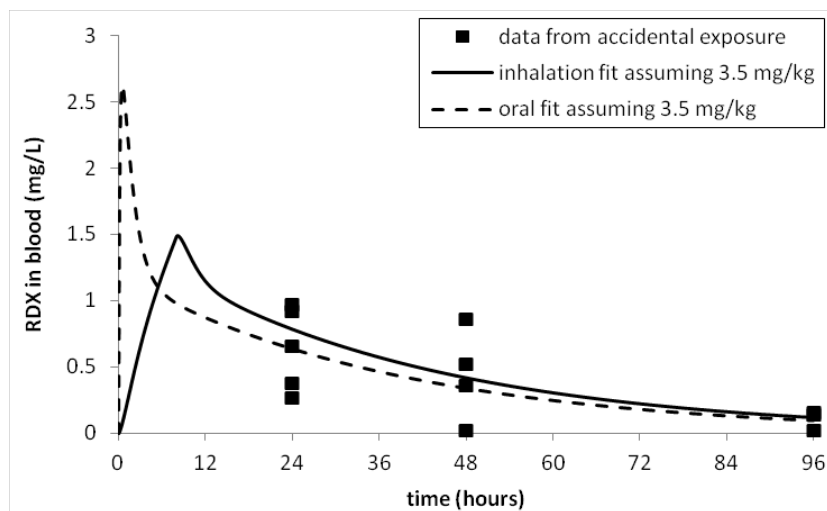
³A constant drinking water ingestion rate interspaced between episodes of no ingestion was assumed. Each 12-hour awake period consisted of eight cycles that alternated between 1.5-hour cycles of frequent sipping (continuous ingestion) and zero ingestion for 45 minutes each. Each 12-hour sleeping period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

1 Figure C-10. The model predictions for the [Özhan et al. \(2003\)](#) data using the best fit values of an
 2 oral dose = 3.5 mg/kg, KAS = 1.75/hour, and KfC = 9.87 kg^{0.33}/hour are shown in Figure C-11.
 3



4
 5 The best fit values were KAS = 1.75/hour, dose = 58.9 mg/kg, and KfC = 9.87 kg^{0.33}/hour.

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



9
 10 For an assumed oral exposure, the best fit values were KAS = 1.75/hour, dose = 3.5 mg/kg, and
 11 KfC = 9.87 kg^{0.33}/hour. For the same 3.5 mg/kg dose and metabolism rate constant, an inhalation
 12 exposure found a best fit value for Klung of 0.75/hour.

13 **Figure C-11. EPA human model predictions fitted to observed RDX blood**
 14 **concentrations resulting from accidental exposure to adults assumed to be**
 15 **70 kg ([Özhan et al., 2003](#)).**

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

21 ***Sensitivity Analysis of the Rat and Human PBPK Models***

22 A sensitivity analysis was performed to see how each model parameter affects the model
23 output. A sensitivity coefficient, defined as the change in a specified dose metric due to a 1%
24 increase in the value of a parameter, was calculated for each parameter in the rat and human
25 models. This analysis was carried out for both short-term (24 hours following a single oral dose of
26 1.5 mg/kg RDX) and longer-term (90 days of repeated oral dosing with 1.5 mg/kg RDX) exposures
27 for the dose-metric of blood AUC. Parameters with sensitivity coefficients >0.1 in absolute value
28 (i.e., considered sensitive) are presented in Table C-7. For the blood AUC dose-metric, the only
29 sensitive RDX-specific parameter is the K_{fC}. This sensitivity is likely because bioavailability was
30 assumed to be 100% and metabolism is the only route of elimination in the model. These
31 assumptions mean that all administered RDX will be absorbed and metabolized; in other words, the
32 blood AUC is proportional to the dose and inversely proportional to the metabolic clearance rate
33 constant. For the parameter values in this model, the rate of metabolism is relatively slow
34 compared to the transport of RDX between other tissues and the site of metabolism in the liver, so
35 that the blood AUC is not sensitive to parameters that impact transport such as K_{QC} and K_{QL}.
36 Because the metabolic clearance rate constant is scaled to body weight and by liver volume, the
37 blood AUC is also sensitive to these parameters. The sensitivity analysis by [Sweeney et al. \(2012b\)](#)

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

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

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

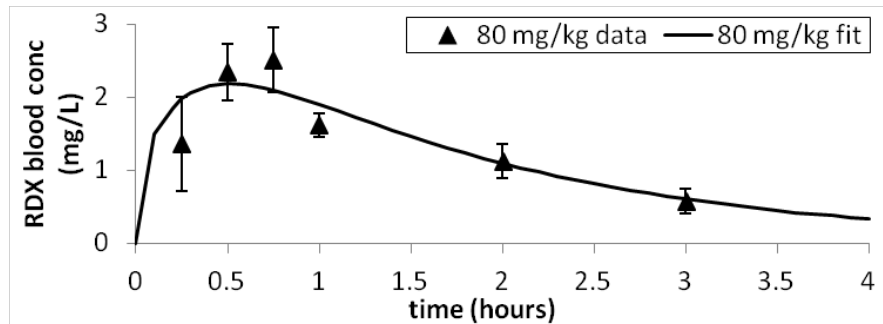
4
 5 Parameters with sensitivity coefficients <0.1 in absolute value are considered not sensitive, and are listed below:
 6 • cardiac output (KQC);
 7 • fractional blood flow to all tissues (liver, KQL; fat, KQF; slowly perfused tissues, KQS; brain, KQB)
 8 • fractional tissue volume of fat (KVF), brain (KVB), and blood volume (KVV)
 9 • blood partition coefficients to all tissues (liver, PL; fat, PF; rapidly perfused, PR; slowly perfused, PS; brain, PB)
 10 • absorption rates from GI (KAS, KT, KAD)
 11

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

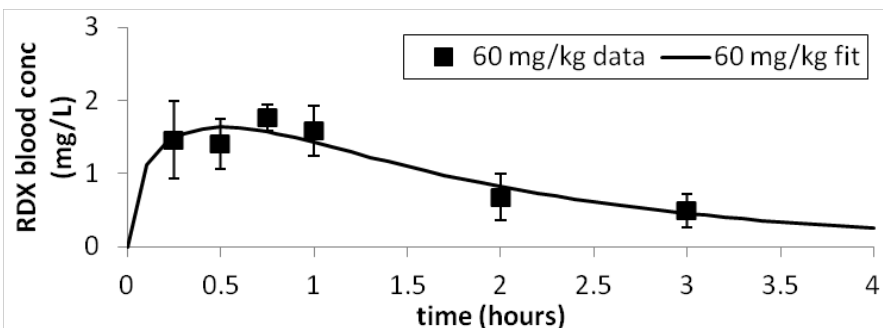
21 ***Simulating Exposures in Mice***

22 Physiological parameters specific to mice were obtained from the literature ([Brown et al.,](#)
 23 [1997](#)) and are shown in Table C-5. The partition coefficients calculated for mice by [Sweeney et al.](#)
 24 [\(2012b\)](#) were used, and include the liver, brain, and richly perfused tissues. The partition
 25 coefficients for the fat and slowly perfused tissues from the [Sweeney et al. \(2012b\)](#) mouse model
 26 were not used because they were estimated via optimization of fits to rat i.v. data. Instead, the
 27 partition coefficient for fat tissues was set equal to the value calculated by [Krishnan et al. \(2009\)](#) for
 28 rat fat tissue, 7.55. The partition coefficient for slowly perfused tissues (0.9) was calculated for
 29 mouse tissues using the same methodology as [Krishnan et al. \(2009\)](#). The rate constants for oral
 30 absorption and metabolism were optimized to fit the data from [Sweeney et al. \(2012b\)](#) for mouse
 31 blood RDX concentrations. The model predictions were in good agreement with the RDX blood
 32 concentrations reported by [Sweeney et al. \(2012b\)](#), as shown in Figure C-12.

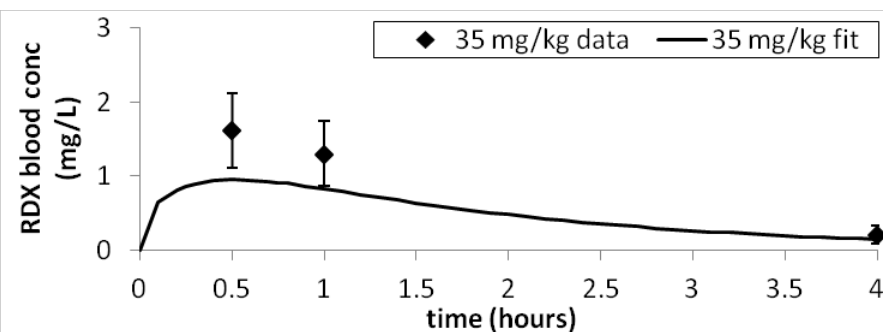
1



2



3



4

5 Model fits and mean and standard deviation of observed RDX blood concentrations in female
 6 B6C3F₁ mice (0.0205 kg) for doses of 35, 60, and 80 mg/kg with KAS = 0.6/hour and
 7 KfC = 77 kg^{0.33}/hour. Experimental data from [Sweeney et al. \(2012b\)](#).

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

10

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

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

2
3 where $\Delta\text{blood concentrations}$ are the successive blood concentrations, Δt is the time
4 between measured concentrations, and K_{el} is the terminal elimination rate constant
5 (calculated from the slope of the linear regression line to the log of blood
6 concentrations)

7
8 For the mouse data from [Sweeney et al. \(2012b\)](#) for the doses of 35, 60, and 80 mg/kg, the
9 results of the AUC_{total} calculation are 3.35, 3.70, and 4.75 mg/L hour; normalized to the
10 administered dose, these are 0.096, 0.062, and 0.059 mg/L hour per mg/kg. For the blood
11 concentrations measured in rats in the [Krishnan et al. \(2009\)](#) study (Figure C-3), the animals
12 received a single oral (gavage) dose of RDX dissolved in water similar to the [Sweeney et al. \(2012b\)](#)
13 study. The [Krishnan et al. \(2009\)](#) study used doses of 1.53 and 2.07 mg/kg and the results of the
14 AUC_{total} calculation are 6.1 and 11.9 mg/L hour. Including the extrapolation of the blood
15 concentration from the last time point with the terminal elimination rate constant, K_{el} had a major
16 contribution to the AUC_{total} (approximately one-third), which adds uncertainty to the result, so the
17 AUC_{total} was also calculated without this term and the results are 4.1 and 7.5 mg/L hour. The
18 AUC_{total} values normalized to the administered doses are 4.0 and 5.8 mg/L hour per mg/kg
19 (including the extrapolation from the last time point) or 2.7 and 3.6 mg/L hour per mg/kg
20 (excluding the extrapolation from the last time point). Overall, the AUC_{total} normalized to the
21 administered doses for the rat are of the order 10–100 times greater than for the mouse. This non-
22 compartmental analysis of the data is independent of the PBPK modeling and shows the extent of
23 the toxicokinetic differences for RDX between the mouse and rat.

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

30 ***Rat to Human Extrapolations***

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 steady state values were considered representative of the study and were used. To calculate
2 steady-state values for daily exposure, the simulations were run until the daily average had a <1%
3 change between consecutive days. For both the rat and human PBPK models, both dose metrics
4 correlated linearly with the administered dose. For rats dosed via gavage, the slope of
5 administered dose versus AUC was 6.800 mg/L-day / mg/kg-day and that for C_{\max} was
6 0.4718 mg/L / mg/kg-day. For a continuous dose, the slope of dose versus AUC was the same
7 (6.800 mg/L-day / mg/kg-day) and for C_{\max} was 0.3951 mg/L / mg/kg-day. For humans, assuming
8 a drinking water dose sipping pattern, the slope of administered dose versus AUC was
9 13.95 mg/L-day / mg/kg-day and that for C_{\max} was 0.7316 mg/L / mg/kg-day. Given this linearity
10 in internal metrics and assuming that equal internal metrics in rats and humans are associated with
11 the same degree of response, the HEDs could then be directly determined by multiplying the lower
12 bound on the benchmark dose (BMDL) in rats by the ratio of these slopes. For a gavage dose in rats
13 converted to a human drinking water dose, the ratio for AUC was $6.800 / 13.95 = 0.487$ and C_{\max}
14 was $0.4718 / 0.7316 = 0.645$. For a continuous dose in rats converted to a human drinking water
15 dose, the ratio for AUC was $6.800 / 13.95 = 0.487$ and for C_{\max} was $0.3951 / 0.7316 = 0.540$. These
16 ratios were applied in Table 2-2 to calculate the POD_{HED} from the rat benchmark dose lower
17 confidence limits (BMDLs) and no-observed-adverse-effect levels (NOAELs) for each endpoint.

Mouse to Human Extrapolations

18
19 The mouse and human PBPK models as described above were applied to derive HEDs for
20 candidate PODs for endpoints selected from mouse bioassays. The mouse and human PBPK models
21 were used to estimate two dose metrics—the area under the curve (AUC) and peak concentration
22 (C_{\max}) for RDX concentration in arterial blood. The relationships between administered dose and
23 both internal metrics (AUC and C_{\max}) were evaluated with the mouse PBPK model over the range
24 10 $\mu\text{g}/\text{kg}\text{-day}$ to 100 mg/kg-day and with the human PBPK model over the range 0.05 $\mu\text{g}/\text{kg}\text{-day}$ to
25 200 mg/kg-day, ranges that encompass the PODs. The times to reach steady state for the dose
26 metrics were shorter than the duration of the toxicity studies, so the steady state values were
27 considered representative of the study and were used. To calculate steady state values for daily
28 exposure, the simulations were run until the daily average had a <1% change between consecutive
29 days. For both the mouse and human PBPK models, both dose metrics correlated linearly with the
30 administered dose. For mouse dosed via gavage, the slope of administered dose versus AUC was
31 0.0656 mg/L-day / mg/kg-day and that for C_{\max} was 0.0273 mg/L / mg/kg-day. For a continuous
32 dose, the slope of dose versus AUC was the same 0.0656 mg/L-day / mg/kg-day and for C_{\max} was
33 0.0081 mg/L / mg/kg-day. For humans, assuming a drinking water dose sipping pattern, the slope
34 of administered dose versus AUC was 13.95 mg/L-day / mg/kg-day and that for C_{\max} was
35 0.7316 mg/L / mg/kg-day. Given this linearity in internal metrics and assuming that equal internal
36 metrics in mice and humans are associated with the same degree of response, the HEDs could then
37 be directly determined by multiplying the BMDL in mice by the ratio of these slopes. For a gavage
38 dose in mice converted to a human drinking water dose, the ratio for AUC was $0.0656 / 13.95 =$

1 0.0047 and C_{\max} was $0.0273 / 0.7316 = 0.373$, respectively. For a continuous dose in mice
2 converted to a human drinking water dose, the ratio for AUC was $0.0656 / 13.95 = 0.0047$ and for
3 C_{\max} was $0.0081 / 0.7316 = 0.011$. These ratios were applied in Table 2-2 to calculate the POD_{HED}
4 from the mouse BMDLs and NOAELs for each endpoint.

5 ***Summary of Confidence in PBPK Models for RDX***

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

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

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

- 26 1) RDX is readily metabolized in several species, yet there are no data on the toxicokinetics of
27 RDX metabolites in the rat and human. Some data are available for the n-nitrosoamine
28 metabolites (a minor metabolic pathway) in mice, but the data are too sparse to help better
29 parameterize a PBPK model. Consequently, the PBPK models used in this assessment do
30 not incorporate the kinetics of RDX metabolites.
- 31 2) The available toxicokinetic data are not sufficient to uniquely identify a parameter value for
32 RDX oral bioavailability. Consequently, the model assumes 100% bioavailability even
33 though some studies in rats suggest that a lower bioavailability is likely.
- 34 3) The human model is based on single accidental exposures, and the exposure concentrations
35 are not known.
- 36 4) The only route of clearance of RDX used in the models is that of total metabolism, which
37 appears reasonable for the rat for which only roughly 5% of the RDX was detected

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

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

10 6) The analytical detection limit in the mouse pharmacokinetic study is too high to enable
11 detection at the lower doses. The lowest dose that resulted in a detectable level of RDX in
12 blood was 35 mg/kg; this dose was high enough to manifest some toxicity in the chronic
13 mouse bioassay. The measured blood concentration at the final 4-hour timepoint at the
14 35 mg/kg dose was based on the level measured from one animal only (in the other five
15 animals exposed at this dose, three were non-detects, one was excluded as an outlier, and
16 one animal died). Data from a single animal decreases the confidence in the calibration of
17 the mouse PBPK model.

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

Model Code for RDX PBPK Model Used in the Assessment

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

1 **C.2. HUMAN STUDIES**

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

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference, number of cases, exposure setting	Exposure	Effects observed	Comments
<p>Woody et al. (1986)</p> <p>1 male child (August 1984)</p> <p>Manufacturing</p>	<p>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)</p>	<p>Seizures before and after admission; EEG revealed prominent diffuse slowing that was greatest in the occipital regions with no evidence of epileptiform activity; elevated AST on admission and after 24 hrs; within 24 hrs, the child was extubated and intensive care withdrawn; normal mental status and normal neurological examination at discharge</p>	<p>Mother worked at an explosive plant in which RDX was manufactured in a plasticized form</p>
<p>Goldberg et al. (1992)</p> <p>1 male</p> <p>Nonwartime</p>	<p>Ingestion after chewing a piece (unknown size) of "Semtex" plastic explosive 4 hrs before first seizure</p>	<p>Frontal headache and two tonic-clonic seizures; progressively disseminating petechial rash suggestive of meningococcal infection afebrile; normotensive; no photophobia; no neurological abnormalities; florid petechial rash over the face and trunk; lacerated tongue</p> <p>Initial results included leukocyte count of 10.8×10^9/dL (87% neutrophils); hemoglobin, platelet count, coagulation screen, serum and CSF biochemistry all within normal limits; CSF and blood bacteriologically unremarkable</p> <p>Shortly following admission, headache and rash disappeared; no further seizures</p>	
<p>Harrell-Bruder and Hutchins (1995)</p> <p>1 male</p> <p>Nonwartime</p>	<p>Ingestion of C-4 (chewing on a piece of undetermined size)</p>	<p>Tonic-clonic seizures; postictal state; EEGs were normal; brisk deep tendon reflexes</p>	
<p>Testud et al. (1996a)</p> <p>1 male</p> <p>Manufacturing</p>	<p>Inhalation and possible dermal exposure during the RDX manufacturing process</p>	<p>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</p>	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

- 1
- 2 ALT = alanine aminotransferase; AST = aspartate transaminase; BUN = blood urea nitrogen; CNS = central nervous
- 3 system; CSF = cerebrospinal fluid; EEG = electroencephalogram; WBC = white blood cell

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

- 1
- 2 ^a[Ma and Li \(1993\)](#) describe symptoms reported by subjects during a physical examination, but these are not included in the evidence table because responses
- 3 for individual symptoms were not identified.
- 4
- 5 CI = confidence interval; HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; LOD = limit of detection; TNT = trinitrotoluene

1 C.3. OTHER PERTINENT TOXICITY INFORMATION

2 C.3.1. Mortality in Animals

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

9 Following chronic dietary exposure, an increased rate of mortality in F344 rats, and in
10 particular male rats, at 40 mg/kg-day was largely attributed to RDX-related effects on the kidneys
11 ([Levine et al., 1983](#))⁴; see further discussion in Section 1.2.2. In a comparable chronic study, mice
12 were less sensitive than rats with respect to mortality following RDX exposure. After the high dose
13 was reduced from 175 to 100 mg/kg-day at week 11 in a 2-year dietary study in B6C3F₁ mice
14 because of high mortality, the mortality curve at 100 mg/kg-day in surviving mice was not
15 significantly different from the control group for the duration of the 2-year study ([Lish et al., 1984](#)).
16 The investigators did not identify the probable cause of death at 175 mg/kg-day.

17 Increased rates of mortality were also observed in experimental animals that ingested RDX
18 for durations up to 6 months ([Lish et al., 1984](#); [Levine et al., 1983](#); [Levine et al., 1981a](#); [Cholakis et](#)
19 [al., 1980](#); [von Oettingen et al., 1949](#)). The most detailed data on RDX-related mortality come from a
20 90-day gavage study in F344 rats by [Crouse et al. \(2006\)](#). Across groups of rats exposed to
21 8–15 mg/kg-day RDX, pre-term deaths occurred in male rats as early as day 26–78 and in female
22 rats as early as day 8–16 ([Johnson, 2015](#); [Crouse et al., 2006](#)). Treatment-related mortality was
23 also observed in the dams of rats exposed gestationally by gavage at doses ranging from 20 to
24 120 mg/kg-day ([Angerhofer et al., 1986](#); [Cholakis et al., 1980](#)). Deaths were additionally reported
25 in one of 40 pregnant dams in both 2 and 6 mg/kg-day groups in the rat developmental toxicity
26 study by [Angerhofer et al. \(1986\)](#)

27 In general, the evidence suggests that mortality occurs at lower doses in rats than in mice
28 (e.g., comparison of rates from the 2-year dietary studies in mice by [Lish et al. \(1984\)](#) and in rats by
29 [Levine et al. \(1983\)](#)), and at lower doses following gavage administration than dietary
30 administration (e.g., comparison of rates from the 13-week rat studies using gavage ([Crouse et al.](#)
31 [2006](#)) and dietary ([Levine et al., 1981a](#)) administration). An RDX formulation with a larger particle
32 size (e.g., ~200 µm) ([Cholakis et al., 1980](#)), which could potentially reduce the ability of RDX to

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 enter the bloodstream, appears to produce less mortality than formulations with finer particle sizes
 2 (e.g., median particle diameter of 20 µm) (Levine et al., 1981a). There is evidence that mortality
 3 may be associated with nervous system effects; several investigators reported that unscheduled
 4 deaths were frequently preceded by convulsions or seizures (Crouse et al., 2006; Levine et al., 1983;
 5 Cholakis et al., 1980). In a number of studies, treatment-related mortality was observed at doses as
 6 low as doses associated with nervous system effects (Crouse et al., 2006; Angerhofer et al., 1986;
 7 Levine et al., 1983; Levine et al., 1981a; Cholakis et al., 1980; von Oettingen et al., 1949). The
 8 evidence for an association between nervous system effects and mortality is discussed in more
 9 detail in Section 1.2.1, Nervous System Effects.

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

13 **Table C-10. Evidence pertaining to mortality in animals^a**

Reference and study design	Results					
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs (mortality incidence also provided for mice through week 11 when the high dose was dropped because of high mortality at that dose, and from the report of the 6-month interim sacrifice)	Doses	0	1.5	7.0	35	175/100
	Mortality at 11 wks (incidence)					
	M	1/85	0/85	0/85	0/85	30/85
	F	0/85	0/85	0/85	0/85	36/85
	Mortality at 6 mo (incidence)					
	M	1/85	2/85	3/85	3/85	34/85
	F	0/85	1/85	0/85	0/85	36/85
	Mortality at 2 yrs (incidence)					
	M	20/65	23/65	25/65	29/65	41/65
	F	16/65	21/65	14/65	21/65	42/65
After the high dose was reduced to 100 mg/kg-d, survival was similar to controls.						
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant: 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs (mortality incidence also provided for mice through week 13, and from the report of the 6-month scheduled sacrifice)	Doses	0	0.3	1.5	8.0	40
	Mortality at 13 wks (incidence)					
	M	0/75	0/75	0/75	0/75	0/75
	F	0/75	0/75	0/75	0/75	0/75
	Mortality in 6-mo interim sacrifice animals (incidence)[#] [#] includes spontaneous death and moribund sacrifice					
	M	0/75	0/75	0/75	0/75	5/75
	F	0/75	0/75	0/75	0/75	0/75
Mortality at 2 yrs (incidence)						

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
	M	17/55	19/55	30/55*	26/55	51/55*	
	F	12/55	10/55	13/55	14/55	27/55*	
Cholakis et al. (1980) Mice, B6C3F ₁ , 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females) ^b Diet 13 wks	Doses	0	79.6	147.8	256.7		
	Mortality (incidence)						
	M	0/10	0/10	0/10	4/10*		
	F	0/11	0/12	0/10	2/12		
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Mortality (incidence)						
	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	1/10 (accidental death)	0/10	0/10	0/10	0/10	0/10
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	Doses	0	5	16	50		
	Mortality in F0 adults (incidence)^c						
	M (F0)	0/22	0/22	0/22	2/22		
	F (F0)	0/22	0/22	0/22	6/22		
M + F (F0)	0/44	0/44	0/44	8/44*			
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Mortality (incidence)						
	M	0/10	0/10	1/10	3/10	2/10	3/10
	F	0/10	0/10	1/10	2/10	5/10	4/10
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)^d Rats, F344, 10/sex/group; 30/sex for control	Doses	0	10	30	100	300	600
	Mortality (incidence)^e						
	M	0/30	0/10	0/10	8/10	10/10	10/10

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	F	0/30	1/10	0/10	5/10	10/10	10/10
von Oettingen et al. (1949) Rats, sex/strain not specified, 20/group 90–97% pure, with 3–10% HMX; particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	Doses	0	15	25	50		
	Mortality (incidence)						
		0/20	1/20 (probably not related to RDX)	8/20	8/20		
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0	0.1	1	10		
	Mortality (incidence)						
	M	0/3	0/3	1/3 (not related to RDX)	0/3		
	F	0/3	0/3	0/3	0/3		
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^f , 3/sex/group Purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10		
	Mortality (incidence)						
	M	0/3	0/3	0/3	0/3		
	F	0/3	0/3	0/3	1/3 (animal exhibited neurological effects; euthanized)		
von Oettingen et al. (1949) Dogs, breed not specified, 5 females/group (control); 7 females/group (exposed) 90–97% pure, with 3–10% HMX; particle not specified 0 or 50 mg/kg-d Diet 6 d/wk for 6 wks	Doses	0	50				
	Mortality (incidence)						
	F	0/5	1/7				
MacPhail et al. (1985) Rats, Sprague-Dawley derived CD, 8–10 males or females/group Purity 84 ± 4.7%; ≤66 µm particle size 0, 1, 3, or 10 mg/kg-d Gavage 30 d	No mortality was reported (incidence data were not provided).						

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
Cholakis et al. (1980) Rabbits, New Zealand White (NZW), 11–12 pregnant females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 0.2, 2.0, or 20 mg/kg-d Diet GDs 7–29	Doses	0	0.2	2.0	20		
	Mortality (incidence)						
	F	0/11	0/11	0/11	0/11	0/12	
Cholakis et al. (1980) Rats, F344, 24–25 females/group 88.6% pure, with 9% HMX and 2.2% water as contaminants 0, 0.2, 2.0, or 20 mg/kg-d Gavage GDs 6–19	Doses	0	0.2	2.0	20		
	Mortality (incidence)						
	F	0/24	0/24	0/24	5/24 (1 rat accidentally killed; removed from analysis)		
Angerhofer et al. (1986) (range-finding study) Rats, Sprague-Dawley, 6 pregnant females/group Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 10, 20, 40, 80, or 120 mg/kg-d Gavage GDs 6–15	Doses	0	10	20	40	80	120
	Mortality (incidence)						
	F	0/6	0/6	0/6	6/6	6/6	6/6
Angerhofer et al. (1986) Rats, Sprague-Dawley, 39–51 mated females/group Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants 0, 2, 6, or 20 mg/kg-d Gavage GDs 6–15	Doses	0	2	6	20		
	Mortality (incidence)						
	F	0/39	1/40	1/40	16/51 Not reported whether deaths at 2 and 6 mg/kg-d related or likely related to RDX exposure		

- 1
- 2 *Statistically significant ($p < 0.05$) based on analysis by the study authors.
- 3 ^aThe 2-year rat study by [Hart \(1976\)](#) was not included in this evidence table because a malfunctioning heating
- 4 system incident resulted in the premature deaths of 59 animals on study days 75–76 across groups, thereby
- 5 confounding mortality findings.
- 6 ^bDoses were calculated by the study authors.
- 7 ^cData for male and female rats were combined for statistical analysis.
- 8 ^d[Levine et al. \(1981a\)](#) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published
- 9 papers ([Levine et al., 1990](#); [Levine et al., 1981b](#)) present subsets of the data provided in the full laboratory report.
- 10 ^eAnimals receiving 300 mg/kg-day died by week 3 of the study; animals receiving 600 mg/kg-day died by week 1 of
- 11 the study.
- 12 ^fThe species of monkey used in this study was inconsistently reported in the study as either *Cynomolgus* (in the
- 13 methods section) or *Rhesus* (in the summary).
- 14
- 15 TWA = time-weighted average

1 **C.3.2. Other Noncancer Effects**

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

Ocular Effects

9 There are no reports of ocular effects in human case reports or epidemiological studies. In
10 experimental animals, evidence of ocular effects comes from cataract findings in one 2-year
11 bioassay. Specifically, the incidence of cataracts was 73% in female F344 rats exposed to
12 40 mg/kg-day RDX in the diet for 2 years, compared with 32% in the control group ([Levine et al.](#)
13 [1983](#)). After 76 weeks of exposure, the incidence of cataracts in female rats at 40 mg/kg-day (23%)
14 was also elevated compared to controls (6%). The incidence of cataracts was not increased in RDX-
15 exposed male rats in the same study ([Levine et al., 1983](#)), and other studies have not observed
16 ocular effects associated with RDX exposure. Only two rats (dose groups not reported) were
17 observed to have mild cataracts in a 90-day study of male and female F344 rats exposed to RDX at
18 doses up to 15 mg/kg-day by gavage; however, the authors noted that these observations are
19 common in F344 rats at 4 months of age and should not be attributed to treatment ([Crouse et al.](#)
20 [2006](#)). Furthermore, cataracts were not observed in male or female F344 rats exposed to
21 40 mg/kg-day RDX by diet for 90 days ([Cholakakis et al., 1980](#)) or in male or female B6C3F₁ mice
22 exposed to RDX in the diet for 2 years at doses up to approximately 100 mg/kg-day ([Lish et al.](#)
23 [1984](#)). A statistically significant increase in the incidence of cataracts in male mice was initially
24 noted by [Lish et al. \(1984\)](#), but was not confirmed when mice used for orbital bleedings were
25 excluded from the analysis, suggesting that the effect was not treatment related. No ocular effects
26 were observed in monkeys exposed by gavage for 90 days at doses up to 10 mg/kg-day ([Martin and](#)
27 [Hart, 1974](#)).

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

Cardiovascular Effects

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

1 Inconsistent observations of cardiovascular effects have been reported in animal studies.
2 An increase in the relative heart-to-body weight ratio was observed at the highest dose tested in
3 B6C3F₁ mice (male: 13%; female: 17%) and F344 rats (male: 22%; female: 15%) following chronic
4 dietary administration of RDX ([Lish et al., 1984](#); [Levine et al., 1983](#)); however, these doses also
5 resulted in reductions in body weight in both males and females. Dose-related decreases in
6 absolute heart weight in rats were reported in some subchronic (dietary) studies ([Levine et al.](#)
7 [1990](#); [Levine et al., 1981a, b](#); [Cholakis et al., 1980](#)), whereas little change or modest increases in
8 absolute heart weight were observed in other subchronic studies in rats or mice ([Crouse et al.](#)
9 [2006](#); [Cholakis et al., 1980](#)). A subchronic study in male dogs reported a 31% increase in absolute
10 heart weight at the highest dose tested (10 mg/kg-day) ([Hart, 1974](#)).

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

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

Musculoskeletal Effects

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

Immune System Effects

31 RDX is structurally similar to various drugs known to induce the autoimmune disorder
32 systemic lupus erythematosus (SLE). Based on the initial identification of three cases of SLE at
33 one U.S. Army munitions plant, further investigation was conducted on a population of
34 69 employees at five U.S. Army munitions plants with potential exposure to RDX ([Hathaway and](#)
35 [Buck, 1977](#)); no additional cases of SLE were identified. Increased WBC counts have been reported

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

4 In animal studies, increased WBC count in female rats following subchronic dietary
5 exposure to RDX was the only dose-related immune effect reported ([Levine et al., 1990](#); [Levine et
6 al., 1981a, b](#)); WBC counts in male rats were unaffected. Conversely, decreased WBC counts were
7 reported in male and female rats in a 2-year study ([Hart, 1976](#)). Changes in spleen weights were
8 observed across studies, but the responses were not consistent and did not appear to be dose-
9 related. For example, in 90-day studies, [Cholakis et al. \(1980\)](#) identified a statistically significant
10 decrease in absolute spleen weight in female F344 rats at 40 mg/kg-day, while [Crouse et al. \(2006\)](#)
11 observed a statistically significant increase in spleen weight at >10 mg/kg-day. Across studies,
12 there was no significant or dose-dependent pattern of response to suggest that the WBC changes
13 reflect RDX-induced immunotoxicity. No dose-related immune effects from oral exposure to RDX
14 were observed in other animal studies, including a 90-day study in F344 rats specifically designed
15 to evaluate immunotoxicity (parameters included evaluation of red blood cell [RBC] and WBC
16 populations, proportion of cell surface markers, cellularity in proportion to organ weight, B and
17 T cells in the spleen, and CD4/CD8 antigens of maturing lymphocytes in the thymus) ([Crouse et al.,
18 2006](#)). Routine clinical and histopathology evaluations of immune-related organs in a two-
19 generation study in rats ([Cholakis et al., 1980](#)) and chronic studies in rats ([Levine et al., 1983](#)) and
20 mice ([Lish et al., 1984](#)) provide no evidence of immunotoxicity associated with oral (dietary)
21 exposure to RDX.

22 In summary, evidence for immunotoxicity associated with RDX exposure is limited to
23 findings from one study of increased WBC counts in female rats ([Levine et al., 1981a, b](#)). Evidence
24 that RDX is not immunotoxic comes from several animal studies, including other repeat-dose oral
25 studies in mice and rats ([Crouse et al., 2006](#); [Lish et al., 1984](#); [Levine et al., 1983](#); [Cholakis et al.,
26 1980](#)).

Gastrointestinal Effects

27 Clinical signs of nausea and/or vomiting have been frequently identified in case reports of
28 accidental or intentional RDX poisonings, and have generally been concurrent with severe
29 neurotoxicity ([Kasuske et al., 2009](#); [Davies et al., 2007](#); [Küçükardali et al., 2003](#); [Hett and Fichtner,
30 2002](#); [Ketel and Hughes, 1972](#); [Knepshield and Stone, 1972](#); [Hollander and Colbach, 1969](#); [Stone et
31 al., 1969](#); [Merrill, 1968](#); [Kaplan et al., 1965](#); [Barsotti and Crotti, 1949](#)) (see Appendix C, Section C.2).
32 In animal studies, vomiting has also been observed following oral exposure in swine (single-dose
33 study) ([Musick et al., 2010](#)), dogs ([Hart, 1974](#)), and monkeys ([Martin and Hart, 1974](#)). One
34 subchronic oral (diet) rat study from the early literature reported congestion of the GI tract at
35 doses also associated with elevated mortality ([von Oettingen et al., 1949](#)); however, none of the
36 subsequent subchronic or chronic animal studies reported histological changes of the GI tract

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

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

Hematological Effects

7 Elevated prevalence odds ratios (ORs) for hematological abnormalities (i.e., neutropenia,
8 low platelet count, or macrocytosis; see Table C-11 for criteria used to define abnormal) were
9 observed in a case-control study of men (24 cases, 199 controls) exposed to RDX in ordnance
10 factories ([West and Stafford, 1997](#)) (see Table C-11). The prevalence OR for an association between
11 RDX exposure and hematological abnormalities was 1.7 (95% confidence interval [CI] 0.7–4.2) for
12 men with >50 hours of low-intensity exposure (based on 22 cases), while the prevalence OR was
13 1.2 (95% CI 0.3–5.3) for men with >50 hours of high-intensity exposure (based on 2 cases). The
14 ORs from this study must be interpreted with caution given the small sample size, wide CIs, and
15 lack of identification of co-exposures. No changes in hematological parameters (including
16 hemoglobin, hematocrit, and reticulocyte count) were observed in a cross-sectional epidemiologic
17 study of 69 workers exposed to RDX by inhalation (RDX exposure range: undetectable
18 [<0.01 mg/m³] to 1.6 mg/m³) ([Hathaway and Buck, 1977](#)). Humans who ingested or inhaled
19 unknown amounts of RDX or C-4 (~91% RDX) for an acute duration displayed temporary
20 hematological alterations, including anemia, decreased hematocrit, hematuria, and
21 methemoglobinemia ([Kasuske et al., 2009](#); [Küçükardali et al., 2003](#); [Knepshield and Stone, 1972](#);
22 [Hollander and Colbach, 1969](#); [Stone et al., 1969](#); [Merrill, 1968](#)). In other case reports, normal blood
23 counts were observed in accidentally exposed individuals ([Testud et al., 1996a](#); [Goldberg et al.,](#)
24 [1992](#); [Woody et al., 1986](#); [Ketel and Hughes, 1972](#); [Kaplan et al., 1965](#)) (see Appendix C,
25 Section C.2).

26 In animals, hematological alterations were observed following oral exposure in chronic and
27 subchronic studies in both sexes of rats (F344 or Sprague-Dawley) and B6C3F₁ mice (see
28 Table C-12). Increases in platelet count were observed in male and female mice and rats in some
29 subchronic and chronic studies at doses ranging from 0.3 to 320 mg/kg-day ([Lish et al., 1984](#);
30 [Levine et al., 1983](#); [Cholakakis et al., 1980](#)); however, changes were generally inconsistent across
31 studies and were not generally dose-dependent. Similarly, decreased hemoglobin levels/anemia
32 were observed in some chronic and subchronic studies ([Levine et al., 1983](#); [Cholakakis et al., 1980](#);
33 [von Oettingen et al., 1949](#)), particularly at doses ≥ 15 mg/kg-day, but trends in hemoglobin levels
34 across studies did not show a consistent relationship with dose. Other hematological parameters,
35 including WBC counts, reticulocyte counts, and hematocrit, showed conflicting results between
36 studies, marginal responses, or inconsistent changes with increasing dose. Other subchronic

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

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

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results			
<p>Hathaway and Buck (1977) (United States) Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate). Analysis limited to whites; 69 exposed to RDX alone and 24 exposed to RDX and HMX; 338 not exposed to RDX, HMX, or TNT. Exposure measures: Exposure determination based on job title and industrial hygiene evaluation. Exposed subjects assigned to two groups: <LOD or ≥0.01 mg/m³ (mean for employees with exposures ≥LOD: 0.28 mg/m³). Effect measures: Hematology tests. Analysis: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and χ² tests for comparison of proportions).</p>	Hematology tests in men (mean; standard deviation not reported)			
			RDX exposed*	
	Test	Referent (n = 237)	Undetected (<LOD) (n = 22)	>0.01 mg/m ³ (n = 45)
	Hemoglobin	15.2	14.7	15.2
	Hematocrit	42	45.6	47
	Reticulocyte count	0.7	0.9	0.7
	*Includes both workers exposed to RDX alone and RDX and HMX. No differences were statistically significant. Similar results in women.			
	Hematology tests in men (prevalence of abnormal values)			
			RDX exposed*	
	Test (abnormal range)	Referent	Undetected (<LOD)	>0.01 mg/m ³
Hemoglobin (<14)	15/237	3/22	4/45	
Hematocrit (<40)	1/237	1/22	1/45	
Reticulocyte count (>1.5)	18/237	3/22	2/45	
*Includes both workers exposed to RDX alone and RDX and HMX. No differences were statistically significant. Similar results in women.				

1

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

Reference and study design	Results					
<i>Ocular effects</i>						
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100
	Cataracts; 103 wks (incidence)^b					
	M	2/47	2/41	0/41	2/37	2/16
	F	2/50	1/37	6/52	0/46	1/26
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Cataracts; 103 wks (incidence)					
	M	8/40	6/39	6/31	8/35	2/6
	F	14/44	4/48	11/44	8/43	22/30*
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	No ocular effects were observed (gross examination of eye was performed in all animals, and microscopic examination was performed in control and 40 mg/kg-d animals).					
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	No ocular effects were observed (ophthalmic examinations were performed in all animals within 1 wk of sacrifice, and microscopic examination of the eye was performed in control and 15 mg/kg-d animals).					
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	No ocular effects were observed (ophthalmoscopic examination was performed at the end of exposure).					

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
<i>Cardiovascular effects</i>							
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100	
	Absolute heart weight; 104 wks (<i>percent change compared to control</i>)						
	M	0%	4%	4%	5%	7%	
	F	0%	1%	5%	2%	–5%	
	Relative heart-to-body weight; 104 wks (<i>percent change compared to control</i>)						
	M	0%	7%	5%	5%	13%*	
	F	0%	0%	6%	4%	17%*	
	Body weight was significantly lower at termination in males and females exposed to 175/100 mg/kg-d (–5 and –19%, respectively).						
	Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Doses	0	1.0	3.1	10	
		Myocardial fibrosis (<i>percent incidence; number not reported</i>)					
M		20%	–	–	5%		
F		5%	–	–	1%		
Endocardial disease (<i>percent incidence; number not reported</i>)							
M		1%	–	–	3%		
F		0%	–	–	0%		
Absolute heart weight; 104 wks (<i>percent change compared to control</i>)							
M		0%	–6%	–2%	–5%		
F		0%	13%	3%	15%		
Relative heart-to-body weight; 104 wks (<i>percent change compared to control</i>)							
M	0%	–2%	4%	1%			
F	0%	23%	13%	27%			
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40	
	Absolute heart weight; 104 wks (<i>percent change compared to control</i>)						
	M	0%	3%	–2%	–2%	1%	
	F	0%	–1%	0%	–4%	–3%	
	Relative heart-to-body weight; 104 wks (<i>percent change compared to control</i>)						
	M	0%	2%	6%	0%	22%	
F	0%	–2%	3%	–1%	15%		

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
<p>Cholakis et al. (1980) Mice, B6C3F₁, 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)^d Diet 13 wks</p>	Doses	0	10	14	20	28	40
	Absolute heart weight (percent change compared to control)						
	M	0%	–	–	–	7%	7%
	F	0%	–	–	–	0%	0%
	Relative heart weight (percent change compared to control)						
	M	0%	–	–	–	6%	0%
	F	0%	–	–	–	–4%	0%
	Doses	0	80	160	320		
	Focal myocardial degeneration (incidence)						
	M [#]	0/10	–	–	–	5/10 ^{*‡}	
	F [†]	0/11	–	–	–	2/11	
	Absolute heart weight (percent change compared to control)						
	M	0%	0%	0%	0%	8%	
	F	0%	0%	0%	0%	8%	
	Relative heart-to-body weight (percent change compared to control)						
	M	0%	0%	–2%	–2%	6%	
	F	0%	0%	–2%	–2%	2%	
[#] Includes one affected and three unaffected animals that died prematurely. [†] Includes one unaffected animal that died prematurely. [‡] Minimal severity in four rats, moderate in one.							
<p>Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks</p>	Doses	0	10	14	20	28	40
	Focal myocardial degeneration, minimal severity (incidence)						
	M	3/10	–	–	–	–	1/10
	F	2/10	–	–	–	–	6/10
	Absolute heart weight (percent change compared to control)						
	M	0%	–	–	–	0%	–8%*
	F	0%	–	–	–	–6%	–11%*
	Relative heart-to-body weight (percent change compared to control)						
	M	0%	–	–	–	3%	0%
	F	0%	–	–	–	–3%	–8%
	Relative heart-to-brain weight (percent change compared to control)						
M	0%	–	–	–	–4%	–10%*	
F	0%	–	–	–	–5%	–11%*	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	No cardiac effects were observed (microscopic examination of heart was performed in randomly selected F2 animals).						
	Doses	0	5	16	50		
	Absolute heart weight (percent change compared to control)						
	F2 M	0%	3.2%	-6.5%	-		
	F2 F	0%	15%	-3.7%	-		
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	Cardiomyopathy (incidence)						
	M	2/10	-	-	-	-	3/8
	F	0/10	-	-	-	-	1/6
	Absolute heart weight (percent change compared to control)						
	M	0%	-2%	-7%	-1%	1%	11%
	F	0%	-2%	0%	8%	7%	6%
	Relative heart-to-body weight (percent change compared to control)						
	M	0%	4%	2%	1%	-1%	8%
	F	0%	-2%	-7%	-6%	-9%	-16%*
Levine et al. (1990); Levine et al. (1981a); Levine et al. (1981b)^e Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	All animals in the 300 and 600 mg/kg-d groups died prior to study termination.						
	Doses	0	10	30	100	300	600
	Chronic focal myocarditis (incidence)						
	M	8/30	8/10	6/10	1/10	1/10	0/10
	F	8/30	3/10	1/10	1/10	1/10	1/9
	Absolute heart weight (percent change compared to control)						
	M	0%	-2%	-10%	-15%	-	-
	F	0%	-3%	0%	-5%	-	-
	Relative heart-to-body weight (percent change compared to control)						
	M	0%	2%	-4%	3%	-	-
F	0%	-2%	0%	-3%	-	-	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results					
von Oettingen et al. (1949) Rats (sex/strain not specified); 20/group Purity and particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	The study authors reported that there were no cardiac effects (microscopic examination of the heart was performed in all rats; data were not shown).					
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0	0.1	1	10	
	Focal hyalinization of the heart (incidence)					
	M	0/3	–	–	0/3	
	F	0/3	–	–	1/3	
	Absolute heart weight (percent change compared to control)					
	M	0%	–	–	31%	
F	0%	–	–	5.7%		
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10	
	Myocarditis (percent change compared to control)					
	M	1/3	–	–	1/3	
	F	0/3	–	–	0/3	
	Absolute heart weight (percent change compared to control)					
	M	0%	7%	–1%	5%	
F	0%	10%	12%	–12%		
<i>Immune effects</i>						
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	No immune effects were observed with routine hematology, clinical chemistry, or histopathology evaluations.					
	Doses	0	1.5	7.0	35	175/100
	WBC count; 105 wks (percent change compared to control)					
	M	0%	–13%	–8%	–16%	–30%
	F	0%	12%	39%*	28%	0%
	Absolute spleen weight; 105 wks (percent change compared to control)					
	M	0%	24%	31%	–10%	–28%
	F	0%	4%	15%	–17%	16%
	Relative spleen weight; 105 wks (percent change compared to control)					
	M	0%	26%	32%	–11%	–21%
F	0%	4%	15%	–17%	44%	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
	Doses	0	1.0	3.1	10		
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	WBC count; 104 wks (percent change compared to control)						
	M	0%	-13%	-22%*	-34%*		
	F	0%	5%	-32%*	-12%		
	Absolute spleen weight; 104 wks (percent change compared to control)						
	M	0%	-11%	-16%	-4%		
	F	0%	58%	8%	37%		
	Relative spleen weight; 104 wks (percent change compared to control)						
M	0%	-11%	-14%	1%			
F	0%	77%	19%	55%			
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	No immune effects were observed with routine hematology, clinical chemistry, or histopathology evaluations.						
	Doses	0	0.3	1.5	8.0	40	
	WBC count; 105 wks (percent change compared to control)						
	M	0%	-11%	103% ^f	184% ^f	15%	
	F	0%	7%	12%	354% ^f	251% ^f	
	Absolute spleen weight; 105 wks (percent change compared to control)						
	M	0%	5%	-10%	-32%	-49%	
F	0%	-28%	-44%	-35%	17%		
Relative spleen weight; 105 wks (percent change compared to control)							
M	0%	9%	4%	-29%	-38%		
F	0%	-34%	-45%	-36%	9%		
Cholakis et al. (1980) Mice, B6C3F ₁ , 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	Absolute spleen weight (percent change compared to control)						
	M	0%	-	-	-	18%	13%
	F	0%	-	-	-	-2%	-8%
	Relative spleen weight (percent change compared to control)						
M	0%	-	-	-	24%	14%	
F	0%	-	-	-	-3%	-5%	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
Experiment 2: 0, 40, 60, 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females) ^d Diet 13 wks	Doses	0	80	160	320		
	WBC count (percent change compared to control)						
	M	0%	-27%	-12%	30%		
	F	0%	-17%	3%	-3%		
	Absolute spleen weight (percent change compared to control)						
	M	0%	17%	0%	-17%		
	F	0%	-22%	0%	0%		
	Relative spleen weight (percent change compared to control)						
	M	0%	25%	5%	0%		
	F	0%	-12%	0%	-3%		
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	WBC count (percent change compared to control)						
	M	0%	-	-	-	-12%	7%
	F	0%	-	-	-	17%	30%
	Absolute spleen weight (percent change compared to control)						
	M	0%	-	-	-	2%	-4%
	F	0%	-	-	-	-10%	-12%*
	Relative spleen weight (percent change compared to control)						
	M	0%	-	-	-	5%	5%
	F	0%	-	-	-	-8%	-8%
Cholakis et al. (1980) Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d Diet F0 exposure: 13 wks pre-mating, and during mating, gestation, and lactation of F1; F1 exposure: 13 wks after weaning, and during mating, gestation, and lactation of F2; F2 exposure: until weaning	No immune effects were observed upon routine histopathology evaluation.						

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	No effects were observed on thymus or spleen histology, RBC or WBC populations, or lymphocyte populations.						
	Doses	0	4	8	10	12	15
	WBC count (percent change compared to control)						
	M	0%	-5%	-12%	-7%	1%	-3%
	F	0%	22%	45%	12%	52%	29%
	Absolute spleen weight (percent change compared to control)						
	M	0%	-3%	-6%	3%	1%	5%
	F	0%	1%	8%	23%*	17%*	24%*
	Relative spleen weight (percent change compared to control)						
	M	0%	3%	4%	7%	-1%	2%
	F	0%	1%	0%	6%	-1%	-2%
	Absolute thymus weight (percent change compared to control)						
	M	0%	-1%	3%	-10%	-12%	-25%
	F	0%	-7%	12%	19%	32%	19%
Relative thymus weight (percent change compared to control)							
M	0%	-1%	3%	-10%	-12%	-25%	
F	0%	-7%	4%	4%	12%	-6%	
Levine et al. (1990) ; Levine et al. (1981a) ; Levine et al. (1981b) ^e Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all of the rats died before the 13-wk necropsy.						
	Doses	0	10	30	100	300	600
	WBC count (percent change compared to control)						
	M	0%	4%	7%	15%	-	-
	F	0%	23%*	24%*	62%*	-	-
	Absolute spleen weight (percent change compared to control)						
	M	0%	-11%	-16%	-34%	-	-
	F	0%	2%	12%	0%	-	-
	Relative spleen weight (percent change compared to control)						
	M	0%	-9%	-12%	-21%	-	-
F	0%	2%	12%	3%	-	-	

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results				
von Oettingen et al. (1949) Rats, sex/strain not specified, 20/group 90–97% pure, with 3–10% HMX; particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	Doses	0	15	25	50
	WBC count (percent change compared to control)				
	M	0%	–30%	7%	–6%
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0	0.1	1	10
	WBC count (percent change compared to control)				
	M	0%	5%	2%	–19%
	F	0%	–2%	24%	6%
	Absolute spleen weight (percent change compared to control)				
	M	0%	–	–	123%
F	0%	–	–	–11%	
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10
	WBC count (percent change compared to control)				
	M	0%	–32%	0%	–3%
F	0%	–38%	–1%	–41%	
<i>Gastrointestinal effects</i>					
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	No GI tract effects were observed as clinical signs or on gross pathology or histopathology examination.				
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	No GI tract effects were observed as clinical signs or on gross pathology or histopathology examination.				

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	No GI tract effects were observed on gross pathology or histopathology examination. Increased salivation and blood stains around the mouth were noted (affected doses and incidences were not reported); it is not clear whether these effects occurred in animals also experiencing convulsions.						
von Oettingen et al. (1949) Rats (sex/strain not specified); 20/group 90–97% pure, with 3–10% HMX; particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	Congestion of the GI tract was observed in 50 and 100 mg/kg-d rats that also exhibited mortality (40%) and severe neurotoxicity.						
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Vomiting was observed more frequently in the 1 and 10 mg/kg-d groups compared to the control or 0.1 mg/kg-d groups, although some episodes occurred during the intubation procedure.						
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Some nausea and vomiting were reported (incidences and affected dose groups were not reported).						
<i>Hematological effects</i>							
Lish et al. (1984) Mice, B6C3F ₁ , 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 μm 0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality) Diet 2 yrs	Doses	0	1.5	7.0	35	175/100	
	RBC count; 105 wks (percent change compared to control)						
	M	0%	–4%	3%	–3%	14%	
	F	0%	4%	–7%	5%	3%	
	Hemoglobin; 105 wks (percent change compared to control)						
	M	0%	–6%	3%	–5%	9%	
	F	0%	2%	–7%	3%	1%	
	Hematocrit; 105 wks (percent change compared to control)						
	M	0%	–4%	3%	–4%	9%	
	F	0%	3%	–6%	3%	1%	
Platelets; 105 wks (percent change compared to control)							
M	0%	33%	9%	21%	27%		

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results					
	F	0%	-14%	-7%	1%	5%
Hart (1976) Rats, Sprague-Dawley, 100/sex/group Purity and particle size not specified 0, 1.0, 3.1, or 10 mg/kg-d Diet 2 yrs	Doses	0	1.0	3.1	10	
	RBC count; 104 wks (percent change compared to control)					
	M	0%	3%	7%	-2%	
	F	0%	-14%	7%	2%	
	Reticulocyte count; 104 wks (percent change compared to control)					
	M	0%	250%	500%*	850%*	
	F	0%	180%*	-40%	20%	
	Hemoglobin; 104 wks (percent change compared to control)					
	M	0%	3%	4%	0%	
	F	0%	-1%	1%	-2%	
Levine et al. (1983) Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm 0, 0.3, 1.5, 8.0, or 40 mg/kg-d Diet 2 yrs	Doses	0	0.3	1.5	8.0	40
	Hemoglobin levels; 105 wks (percent change compared to control)					
	M	0%	6%	6%	3%	-13%
	F	0%	-5%	1%	-9%	-14%
	RBC count; 105 wks (percent change compared to control)					
	M	0%	5%	2%	-1%	-9%
	F	0%	-2%	2%	-9%	-13%
	Platelet count; 105 wks (percent change compared to control)					
	M	0%	6%	-4%	-10%	-7%
	F	0%	14%	-4%	5%	22%
Hematocrit; 105 wks (percent change compared to control)						
M	0%	5%	5%	2%	-7%	
F	0%	-5%	0%	-8%	-12%	
Cholakis et al. (1980) Mice, B6C3F ₁ , 10–12/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 80, 60, or 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females) ^d Diet 13 wks	Doses	0	80	160	320	
	RBC count (percent change compared to control)					
	M	0%	-5%	-12%*	-2%	
	F	0%	-10%	-1%	1%	
	Reticulocytes (percent change compared to control)					
	M	0%	-36%	-13%	15%	
	F	0%	21%	25%	-19%	
Hematocrit (percent change compared to control)						
M	0%	-1%	-6%	0%		

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
		F	0%	-8%	2%	1%	
	Hemoglobin (percent change compared to control)						
	M	0%	-2%	-7%*	-3%		
	F	0%	-5%	4%	1%		
	Platelets (percent change compared to control)						
	M	0%	33%	28%	22%		
	F	0%	3%	9%	39%		
Cholakis et al. (1980) Rats, F344, 10/sex/group 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size 0, 10, 14, 20, 28, or 40 mg/kg-d Diet 13 wks	Doses	0	10	14	20	28	40
	RBC count (percent change compared to control)						
	M	0%	-	-	-	3%	-1%
	F	0%	-	-	-	-1%	-7%
	Hemoglobin (percent change compared to control)						
	M	0%	-	-	-	2%	-1%
	F	0%	-	-	-	-1%	-1%
	Platelet (percent change compared to control)						
	M	0%	-	-	-	11%	16%*
	F	0%	-	-	-	-23%	-13%
	Reticulocytes (percent change compared to control)						
	M	0%	-	-	-	26%	76%*
	F	0%	-	-	-	-2%	17%
	Hematocrit (percent change compared to control)						
	M	0%	-	-	-	3%	0%
	F	0%	-	-	-	0%	-2%
Crouse et al. (2006) Rats, F344, 10/sex/group 99.99% pure 0, 4, 8, 10, 12, or 15 mg/kg-d Gavage 13 wks	Doses	0	4	8	10	12	15
	RBC count (percent change compared to control)						
	M	0%	1%	-7%	-2%	-4%	-5%
	F	0%	3%	3%	-1%	2%	-2%
	Hemoglobin (percent change compared to control)						
	M	0%	-1%	-5%	0%	-1%	-6%
	F	0%	2%	4%	-1	4%	-4%
	Platelet count (percent change compared to control)						
	M	0%	21%	11%	13%	-8%	34%
	F	0%	6%	40%	47%	34%	-36%

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results						
	Hematocrit (percent change compared to control)						
	M	0%	2%	-5%	0%	-1%	-4%
	F	0%	3%	4%	0%	4%	-2%
Levine et al. (1990) ; Levine et al. (1981a) ; Levine et al. (1981b) ^e Rats, F344, 10/sex/group; 30/sex for control 84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm 0, 10, 30, 100, 300, or 600 mg/kg-d Diet 13 wks	Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all of the rats died before the 13-wk necropsy.						
	Doses	0	10	30	100	300	600
	Hematocrit (percent change compared to control)						
	M	0%	-2%	-1%	-5%	-	-
	F	0%	0%	-4%	-7%	-	-
	Hemoglobin (percent change compared to control)						
	M	0%	-3%	-1%	-6%	-	-
	F	0%	0%	-4%	-8%*	-	-
	RBC count (percent change compared to control)						
	M	0%	-2%	-2%	-5%	-	-
	F	0%	-1%	-4%	-5%	-	-
	Reticulocytes (percent change compared to control)						
	M	0%	-4%	10%	28%	-	-
F	0%	9%	73%	71%	-	-	
von Oettingen et al. (1949) Rats, sex/strain not specified, 20/group 90–97% pure, with 3–10% HMX; particle size not specified 0, 15, 25, or 50 mg/kg-d Diet 13 wks	Doses	0	15	25	50		
	RBC count (percent change compared to control)						
	M + F	0%	-23%	-12%	-14%		
	Hemoglobin (percent change compared to control)						
	M + F	0%	-25%	-7%	-11%		
Hart (1974) Dogs, Beagle, 3/sex/group Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food; purity and particle size not specified 0, 0.1, 1, or 10 mg/kg-d Diet 13 wks	Doses	0	0.1	1	10		
	RBC count (percent change compared to control)						
	M	0%	-3%	3%	2%		
	F	0%	13%	7%	11%		
	Reticulocyte count (percent change compared to control)						
	M	0%	-66%	0%	-50%		
	F	0%	-17%	-50%	0%		
	Hematocrit (percent change compared to control)						
	M	0%	-4%	2%	0%		
	F	0%	6%	1%	7%		

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Reference and study design	Results				
	Hemoglobin (percent change compared to control)				
	M	0%	5%	-2%	0%
	F	0%	8%	-2%	8%
	Histopathological examination revealed increased numbers of degenerate or necrotic megakaryocytes in all bone marrow sections.				
Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks	Doses	0	0.1	1	10
	RBC count (percent change compared to control)				
	M	0%	-3%	2%	-3%
	F	0%	0%	-1%	2%
	Reticulocyte count (percent change compared to control)				
	M	0%	-33%	-50%	-50%
	F	0%	-18%	-36%	45%
	Hematocrit (percent change compared to control)				
	M	0%	-7%	-4%	-1%
	F	0%	10%	7%	3%
	Hemoglobin (percent change compared to control)				
	M	0%	-10%	-8%	-6%
	F	0%	6%	6%	3%

- 1
- 2 *Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
- 3 ^aNo musculoskeletal evidence is presented in this table as no animal study reported effects on the musculoskeletal
- 4 system and all human effects were in case reports (see summary in Appendix C, Section C.2).
- 5 ^bIncidence counts exclude individuals from which blood was obtained via the orbital sinus.
- 6 ^cThe species of monkey used in this study was inconsistently reported in the study as either Cynomolgus (in the
- 7 Methods section) or Rhesus (in the Summary).
- 8 ^dDoses were calculated by the study authors.
- 9 ^e[Levine et al. \(1981a\)](#) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published
- 10 papers ([Levine et al., 1990](#); [Levine et al., 1981b](#)) present subsets of the data provided in the full laboratory report.
- 11 ^fStandard deviations accompanying the mean response in a given dose group were high, suggesting uncertainty in
- 12 the accuracy of the reported percent change compared to control.
- 13
- 14 Note: A dash (“-”) indicates that the study authors did not measure or report a value for that dose group.

1 **C.3.3. Genotoxicity**

2 **RDX**

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

15 RDX has produced negative results in all reverse mutation assays in *S. typhimurium* that use
16 standard levels of the metabolic activation system (S9). No evidence of reverse mutation was
17 observed in *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), either with or
18 without the addition of S9 metabolic activating mixture ([Neuwoehner et al., 2007](#); [George et al.,
19 2001](#); [Lachance et al., 1999](#); [Tan et al., 1992](#); [Cholakis et al., 1980](#); [Whong et al., 1980](#); [Cotruvo et al.,
20 1977](#); [Simmon et al., 1977](#)). One exception is a finding of weak mutagenic activity of RDX to
21 *S. typhimurium* strain TA97a (mutagenicity index = 1.5–2.0) ([Pan et al., 2007a](#)). However, this assay
22 used a high percentage of S9 fraction (9% instead of 4%), indicating that extensive metabolic
23 activation is needed to elicit a mutagenic response.

24 RDX did not cause gene recombination in *S. cerevisiae* strain D3 at concentrations up to
25 23 $\mu\text{g}/\text{mL}$, with or without metabolic activation ([Cotruvo et al., 1977](#); [Simmon et al., 1977](#)).
26 [Simmon et al. \(1977\)](#) noted that the negative findings should be considered in the context of the low
27 concentrations tested. RDX was negative in assays with *S. choleraesius* and *E. coli* with and without
28 metabolic activation ([Neuwoehner et al., 2007](#)). Similarly, RDX did not induce forward mutations
29 (HGPRT locus) in V79 Chinese hamster lung cells, with or without metabolic activation, although
30 minimal cytotoxicity was observed at 180 μM ([Lachance et al., 1999](#)). However, RDX produced
31 revertants in two of three trials in the Mutatox assay with the bacterium *Vibrio fischeri* when tested
32 at doses up to 2.5 $\mu\text{g}/\text{tube}$, with and without S9 ([Arfsten et al., 1994](#)). In the presence of S9, a dose-
33 response was observed; in the absence of S9, no dose-response relationship was detected ([Arfsten
34 et al., 1994](#)). RDX did not induce forward mutations in mouse lymphoma L5178Y cells with or
35 without metabolic activation ([Reddy et al., 2005](#)). During an accompanying range-finding study,
36 precipitates occurred at doses ≥ 250 $\mu\text{g}/\text{mL}$, suggesting that concentrations of RDX in DMSO
37 reported beyond 250 $\mu\text{g}/\text{mL}$ may not be accurate.

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Endpoint	Test system	Dose/ concentration ^a	Results ^b		Comments	Reference
			Without activation	With activation		
Reverse mutation (<i>umu</i> test)	<i>Salmonella choleraesius subsp. chol.</i> (prior <i>S. typhimurium</i>) TA1535/pSK1002	20.6 µg/mL	–	–	No observed effect concentration; tested at highest concentration where the induction rate was below 1.5 for the first time and the growth factor was below 0.5	Neuwoehner et al. (2007)
Reverse mutation (NM2009 test)	<i>S. choleraesius subsp. chol.</i> 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 <i>sfiA</i> gene (SOS chromotest)	<i>Escherichia coli</i> 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	<i>S. typhimurium</i> , TA98, TA100	24.8 µg/mL	–	–	No observed effect concentration; metabolic activation with S9	Neuwoehner et al. (2007)
Reverse mutation	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> 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	<i>Saccharomyces cerevisiae</i> D3	23 µg/mL	–	–	Study authors concluded that this microorganism did not appear to be useful for detecting mutagenicity in several compounds tested	Simmon et al. (1977)
Recombination induction	<i>S. cerevisiae</i> D3	30.8 µg/mL	–	–	Metabolic activation with S9	Cotruvo et al. (1977)

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1
2
3

^aLowest effective dose for positive results; highest dose tested for negative results.

^b+ = positive; ± = equivocal or weakly positive; – = negative.

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

13 **Metabolites of RDX**

14 Several metabolites of RDX, N-nitroso derivatives of the parent compound (mononitroso,
15 dinitroso, and trinitroso compounds, abbreviated MNX, DNX, and TNX, respectively) ([Musick et al.,
16 2010](#); [Major et al., 2007](#)) have been tested directly for genotoxicity ([Pan et al., 2007a](#); [George et al.,
17 2001](#); [Snodgrass, 1984](#)). Miniature pigs were used to detect these trace metabolites because the
18 swine model of the GI tract more closely resembles that of humans ([Major et al., 2007](#)); an
19 identification and quantification of RDX metabolites in humans has not been conducted. A
20 summary of the results of in vitro and in vivo genotoxicity studies of metabolites of RDX is
21 presented in Table C-15.

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

36

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

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

2

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

Endpoint	Test system	Dose/ concentration ^a	Results ^b		Comments	Reference
			Without activation	With activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	<i>Salmonella typhimurium</i> TA97a, TA102	22 µg/plate	–	+	Mono and trinitroso metabolites (MNX and TNX); high S9 activation (9%) used	Pan et al. (2007a)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100	500 µg/plate	+	+	Positive in TA100 (but not in TA98) only for TNX; MNX and DNX were negative	George et al. (2001)
Reverse mutation	<i>S. typhimurium</i> 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)

2
3 ^aLowest effective dose for positive results; highest dose tested for negative results; NR = not reported.

4 ^b+ = positive; ± = equivocal or weakly positive; – = negative; ND = not determined.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

9 In summary, RDX is not mutagenic or genotoxic in vitro or in vivo in typical assays used to
10 detect genotoxicity. In two in vitro studies using more sensitive assays and conditions for detecting
11 mutagenicity, RDX was found to be positive. Several studies showed that the N-nitroso metabolites
12 are genotoxic, but the formation and quantification of these metabolites in humans is not known.
13

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

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

D.1. BENCHMARK DOSE MODELING SUMMARY FOR NONCANCER ENDPOINTS

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

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1
2 ^aFor convulsions in [Crouse et al. \(2006\)](#), the incidence rates across doses were determined to be not statistically
3 significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$).
4 The data were combined across sex for this endpoint prior to modeling.

5
6 In addition to the endpoints presented in Table D-1, the combined incidence of seizure and
7 mortality was modeled for [Crouse et al. \(2006\)](#) to determine the effect of possible underestimation
8 of seizures, as discussed in Section 2.1.6. Table D-2 presents the data on this combined incidence.

9 **Table D-2. Convulsion or mortality endpoints from [Crouse et al. \(2006\)](#)**
10 **selected for dose-response modeling for RDX**

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1
2 ^aIncidence was defined for each animal as the presence of convulsion or mortality. The incidence rates across
3 doses for this endpoint were determined to be not statistically significantly different between the males and
4 females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for this
5 endpoint prior to modeling.

6 **D.1.1. Evaluation of Model Fit and Model Selection**

7 For each dichotomous endpoint, BMDS dichotomous models⁵ were fitted to the data using
8 the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square
9 goodness-of-fit test (χ^2 p -value < 0.10 indicates lack of fit). Other factors were also used to assess
10 model fit, such as scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the
11 vicinity of the benchmark response (BMR).

12 From among the models exhibiting adequate fit, the best-fit model was selected for
13 estimation of the BMD. This model selection was conducted in two stage, first from among only the
14 multistage models to determine a representative multistage model, and second from among the
15 representative multistage model and the non-multistage models. In each stage, the BMDL estimates
16 (95% lower confidence limit on the BMD, as estimated by the profile likelihood method) and AIC
17 values of the models considered in that stage were used to make the selection, as follows. If the
18 BMDL estimates were “sufficiently close,” that is, differed by threefold or less, the model selected
19 was the one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the
20 model with the lowest BMDL was selected. The model selected in the second stage was considered
21 the best-fit model.

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

⁵Unless 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 .

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

4 **D.1.2. Modeling Results**

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

7 ***Nervous System Effects***

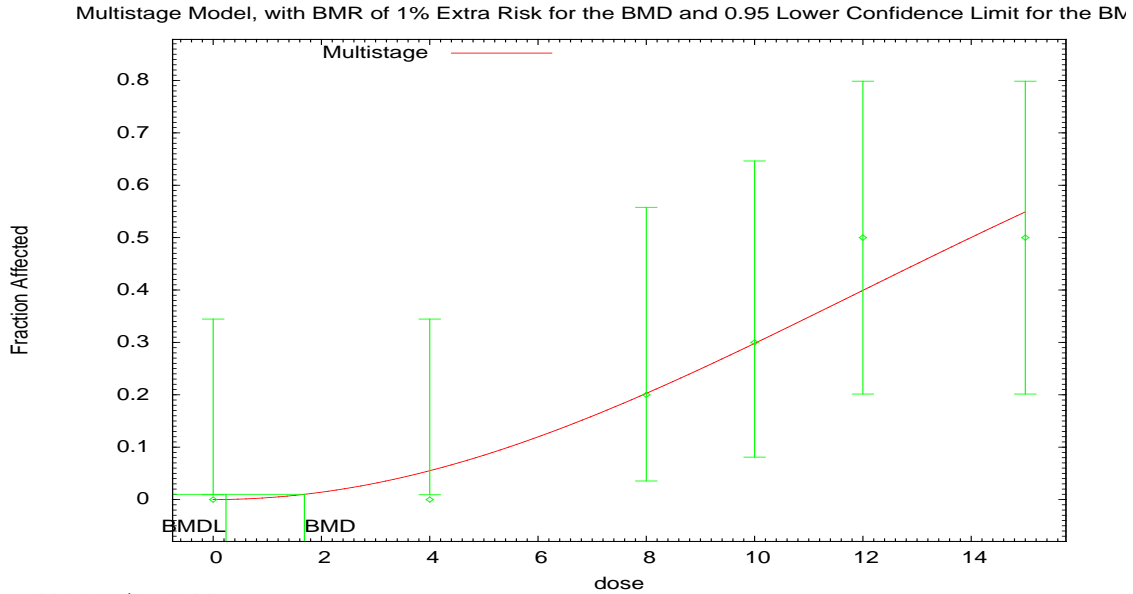
8 Tables D-3 to D-5 (and Figures D-1 to D-3) present the BMD modeling results for incidence
 9 of convulsions for female, male, and male and female F344 rats combined based on data from
 10 [Crouse et al. \(2006\)](#), using BMRs of 10, 5, and 1% extra risk (ER). Table D-6 (and Figure D-4)
 11 present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

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

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

17
 18 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 19 –0.67, 0.14, 0.11, 0.64, and –0.51, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were
 20 5.46 and 2.47 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ values for the selected model were 3.81 and
 21 1.21 mg/kg-day, respectively.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine



1 14:06 02/27 2014

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

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

6 The form of the probability function is: $P[\text{response}] = \text{background} +$
 7 $(1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

8
 9 **Benchmark Dose Computation**

10 BMR = 1% Extra risk

11 BMD = 1.68508

12 BMDL at the 95% confidence level = 0.236479

13
 14 **Parameter Estimates**

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

15
 16 **Analysis of Deviance Table**

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 AIC: = 53.5951

2

3 **Goodness-of-Fit Table**

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

4

5 Chi² = 1.1 d.f. = 5 p-value = 0.9538

6

7 Chi² = 1.16 d.f

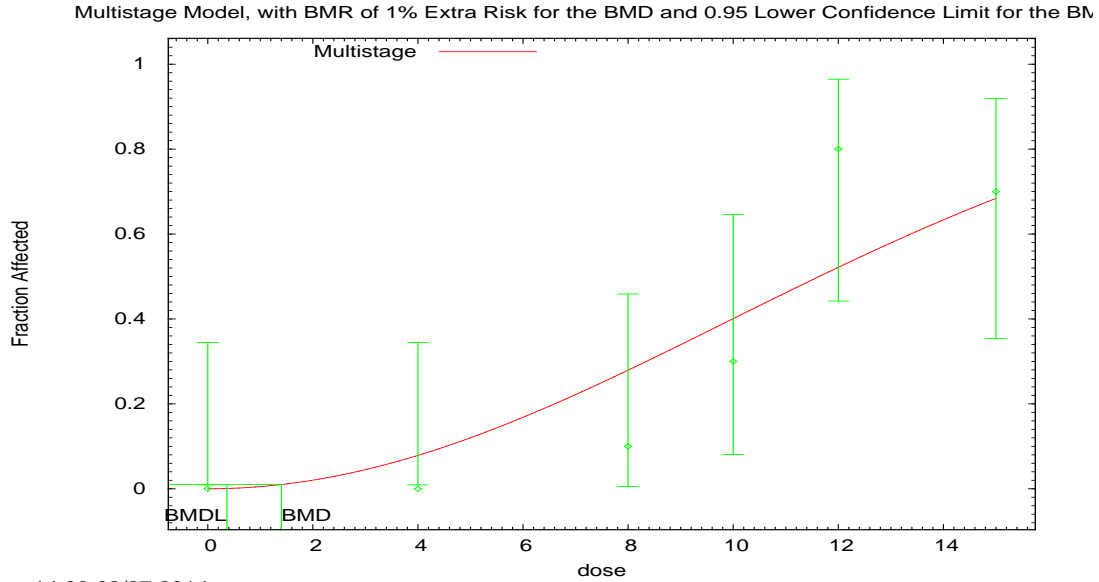
8 **Table D-4. Model predictions for convulsions in male F344 rats exposed to**
 9 **RDX by gavage for 90 days ([Crouse et al., 2006](#)); BMR = 1% ER**

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

10

11 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 12 -0.92, -1.26, -0.65, 1.76, and 0.11, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were
 13 4.54 and 2.95 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ values for the selected model were 3.17 and
 14 1.63 mg/kg-day, respectively.

15 ^bThe Multistage 5° model may appear equivalent to the Multistage 4° model; however, differences exist in digits
 16 not displayed in the table.



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

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

6 **Multistage Model** (Version: 3.3; Date: 02/28/2013)
7 The form of the probability function is: $P[\text{response}] = \text{background} +$
8 $(1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

9
10 **Benchmark Dose Computation**

11 BMR = 1% ER
12 BMD = 1.40125
13 BMDL at the 95% confidence level = 0.363499

14
15 **Parameter Estimates**

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

16

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 **Analysis of Deviance Table**

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

2
3 AIC: = 50.3345

4
5 **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

6
7 Chi² = 5.99 d.f. = 5 p-value = 0.3069
8

1
2

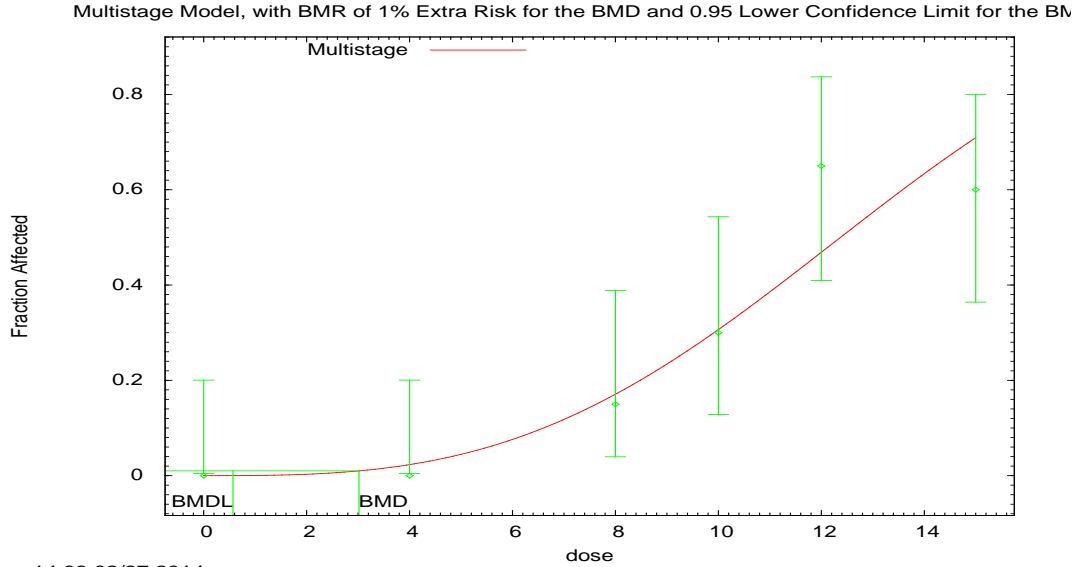
Table D-5. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days ([Crouse et al., 2006](#)); BMR = 1% ER

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

3
4
5
6
7
8
9

^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, -0.69, -0.25, -0.06, 1.62, and -1.08, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were 6.60 and 4.59 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ values for the selected model were 5.19 and 2.66 mg/kg-day, respectively.

^bFor 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.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

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

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

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

Benchmark Dose Computation

BMR = 1% Extra risk
 BMD = 3.01676
 BMDL at the 95% confidence level = 0.569284

Parameter Estimates

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 **Analysis of Deviance Table**

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

2
3 AIC: = 100.913

4
5 **Goodness-of-Fit Table**

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

6
7 Chi² = 4.34 d.f. = 5 p-value = 0.5021

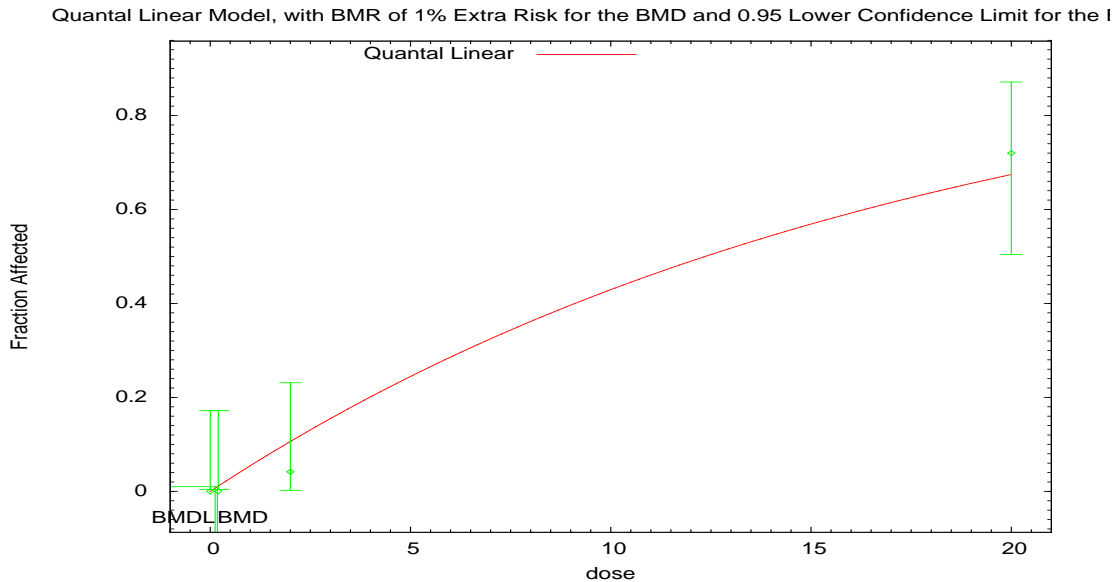
8 **Table D-6. Model predictions for convulsions in female F344 rats exposed to**
9 **RDX by gavage on GDs 6–19 ([Cholakis et al., 1980](#)); BMR = 1% ER**

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

10
11 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-day were 0.00,
12 -0.52, -1.03, and 0.49, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were 1.88 and
13 1.29 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ values for the selected model were 0.915 and
14 0.628 mg/kg-day, respectively.

15 ^bThe Multistage 3[°] model may appear equivalent to the Multistage 2[°] model; however, differences exist in digits
16 not displayed in the table.

1



2

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

3

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

7 **Quantal Linear Model using Weibull Model** (Version: 2.16; Date: 2/28/2013)

8 The form of the probability function is: $P[\text{response}] = \text{background} +$
 9 $(1 - \text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose})]$

10

11 **Benchmark Dose Computation**

12 BMR = 1% ER

13 BMD = 0.179224

14 BMDL at the 95% confidence level = 0.122966

15

16 **Parameter Estimates**

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

17

1 **Analysis of Deviance Table**

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

2
3 AIC: = 42.0769

4
5 **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

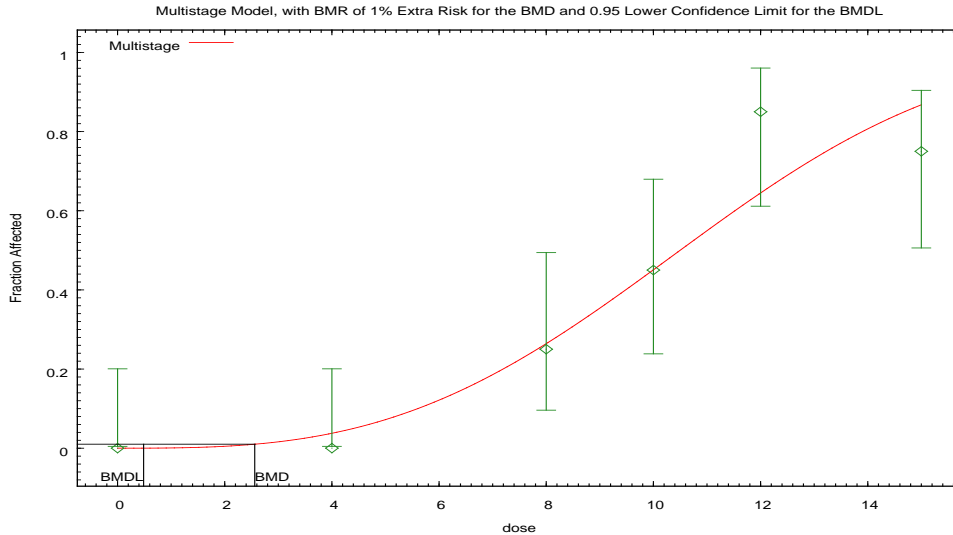
6
7 Chi² = 1.56 d.f. = 3 p-value = 0.6686

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

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

11
12 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0,
13 -0.88, -0.14, -0.01, 1.92, and -1.55, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were
14 5.60 and 3.85 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ values for the selected model were 4.41 and
15 2.25 mg/kg-day, respectively.

1 ^bThe Multistage 4° model may appear equivalent to the Multistage 3° model; however, differences exist in digits
 2 not displayed in the table.
 3 ^cThe Multistage 3° model may appear equivalent to the Multistage 4° model; however, differences exist in digits
 4 not displayed in the table.
 5



6 13:21 01/06 2016
 7 BMR = 1% ER; dose shown in mg/kg-day.

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

12
 13 **Multistage Model** (Version: 3.4; Date: 05/02/2014)
 14 The form of the probability function is: $P[\text{response}] = \text{background} +$
 15 $(1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

16
 17 **Benchmark Dose Computation**
 18 BMR = 1% Extra risk
 19 BMD = 2.56012
 20 BMDL at the 95% confidence level = 0.486284

21
 22 **Parameter Estimates**

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

1
2

Analysis of Deviance Table

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

3
4
5
6

AIC: = 99.1817

Goodness-of-Fit Table

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

7
8
9

Chi² = 6.88 d.f. = 5 p-value = 0.2294

Male Reproductive Effects

Table D-8 (and Figure D-6) presents the BMD modeling results for incidence of testicular degeneration for male B6C3F₁ mice based on data from [Lish et al. \(1984\)](#), using a BMR of 10% ER.

Table D-8. Model predictions for testicular degeneration in male B6C3F₁ mice exposed to RDX by diet for 24 months ([Lish et al., 1984](#)); BMR = 10% ER

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Model ^a	Goodness of fit		BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
LogProbit	0.876	97.564	56.0	16.3	representative multistage model was unnecessary.)
Multistage 2 ^{oc}	0.357	101.10	66.5	35.4	
Multistage 3 ^o					
Multistage 4 ^o					

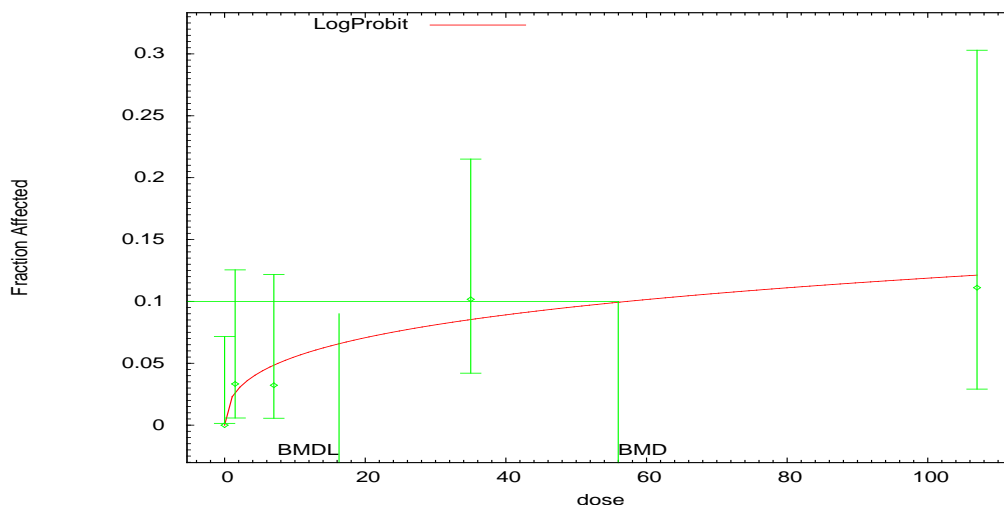
1
2
3
4
5
6
7
8
9
10
11

^aSelected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were 0.00, 0.32, -0.61, 0.43, and -0.17, respectively. The BMD₀₅ and BMDL₀₅ values for the selected model were 7.42 and 0.0477 mg/kg-day, respectively; the BMD₀₁ and BMDL₀₁ values for the selected model were 0.168 and 2.83×10^{-13} mg/kg-day, respectively.

^bFor 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.

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

LogProbit Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the B



12

13:42 02/14 2014

13 **Figure D-6. Plot of incidence rate by dose, with fitted curve for selected model,**
14 **for testicular degeneration in male B6C3F₁ mice exposed to RDX by diet for**
15 **24 months ([Lish et al., 1984](#)).**

16

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

17 The form of the probability function is: $P[\text{response}] = \text{Background} + (1 - \text{Background}) * \text{CumNorm}(\text{Intercept} + \text{Slope} * \text{Log}(\text{Dose}))$, where $\text{CumNorm}(\cdot)$ is the cumulative normal
18 distribution function
19

20 Slope parameter is not restricted
21

22

1 **Benchmark Dose Computation**
 2 BMR = 10% ER
 3 BMD = 55.9784
 4 BMDL at the 95% confidence level = 16.2787

5
 6 **Parameter Estimates**

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

7
 8 **Analysis of Deviance Table**

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

9
 10 AIC: = 97.5635

11
 12 **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

13
 14 Chi^2 = 0.69 d.f. = 3 p-value = 0.8759

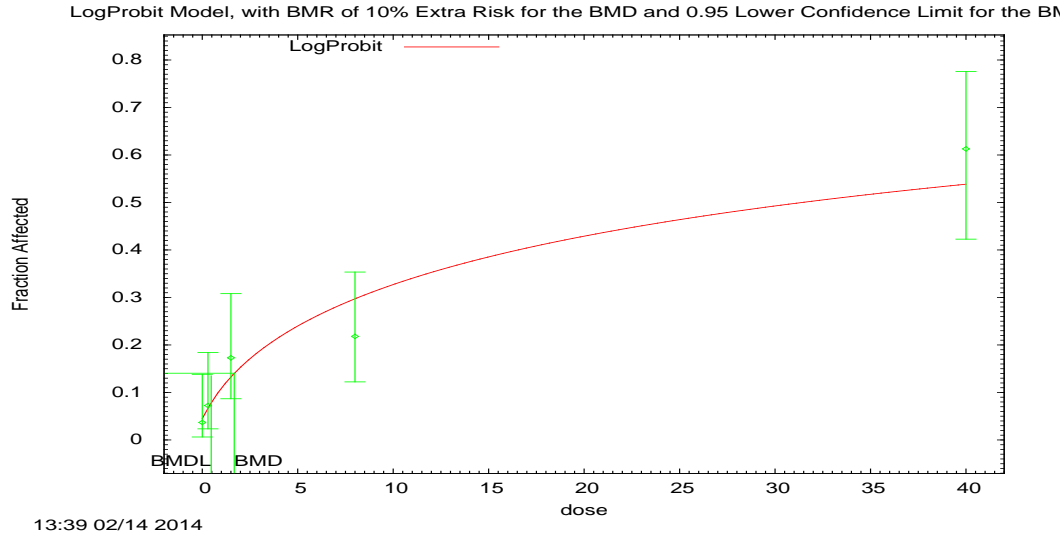
15
 16 **Kidney/Urogenital System Effects**

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

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

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

4
 5 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were
 6 -0.289, 0.172, 0.846, -1.298, and 0.819, respectively. The BMD₀₅ and BMDL₀₅ values for the selected model were
 7 0.702 and 0.122 mg/kg-day, respectively; the BMD₀₁ and BMDL₀₁ values for the selected model were 0.137 and
 8 0.00906 mg/kg-day, respectively.
 9 ^bThe Gamma model had a power parameter estimate of 1 (boundary of parameter space). The Multistage 2°, 3°,
 10 and 4° models had b2, b3, and b4 coefficients of 0 (boundary of parameter space). The models in this row are
 11 equivalent to the Quantal-Linear model.
 12 ^cThe Weibull model may appear equivalent to the Quantal-Linear model; however, differences exist in digits not
 13 displayed in the table.
 14



1

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

5

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

7 The form of the probability function is: $P[\text{response}] = \text{Background} + (1 - \text{Background}) * \text{CumNorm}(\text{Intercept} + \text{Slope} * \text{Log}(\text{Dose}))$, where $\text{CumNorm}(\cdot)$ is the cumulative normal
 8 distribution function
 9

10 Slope parameter is not restricted

11

12 **Benchmark Dose Computation**

13 BMR = 10% ER

14 BMD = 1.67454

15 BMDL at the 95% confidence level = 0.468688

16

17 **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

18

1 **Analysis of Deviance Table**

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

2
3 AIC: = 202.029

4
5 **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

6
7 Chi² = 3.18 d.f. = 2 p-value = 0.2035

8
9 **D.1.3. Mortality: Dose-Response Analysis and BMD Modeling Documentation**

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

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

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

1
2 ^aFor [Levine et al. \(1981a\)](#) and [Crouse et al. \(2006\)](#), the incidence rates across doses were determined to be not
3 statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test
4 ($p \geq 0.10$). The data were combined across sex for each of these endpoints prior to modeling.
5 ^bFor [von Oettingen et al. \(1949\)](#), one mortality was reported in the 15 mg/kg-day dose group. However, this
6 mortality was most likely not related to RDX, so the animal that died was excluded.
7 ^cFor [Cholakis et al. \(1980\)](#), one accidental death was reported in the 0 mg/kg-day dose group. The animal that died
8 was excluded.
9

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

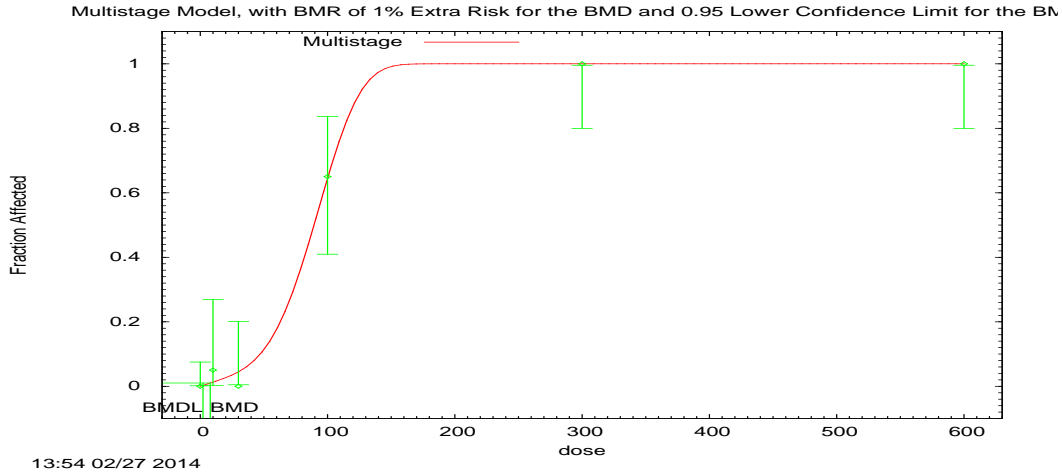
1 and female; mortality in female CD rats and male and female F344 rats from [Cholakis et al. \(1980\)](#);
 2 and mortality in female F344 rats during gestational exposure from [Cholakis et al. \(1980\)](#).

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

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

6
 7 ^aSelected model in bold; scaled residuals for selected model for doses 0, 10, 30, 100, 300, and 600 mg/kg-day were
 8 0.00, 1.48, -0.97, 0.05, 0.00, and 0.00, respectively. The BMD₁₀ and BMDL₁₀ estimates for the selected model
 9 were 47.2 and 22.2 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ estimates for the selected model were
 10 32.4 and 11.0 mg/kg-day, respectively.

11 ^bThe parameter convergence parameter was increased to 2×10^{-8} to obtain convergence.
 12



1

2
3
4

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

5

6

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

7

The form of the probability function is: $P[\text{response}] = \text{background} +$

8

$(1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

9

10

Benchmark Dose Computation

11

BMR = 1% Extra risk

12

BMD = 7.85287

13

BMDL at the 95% confidence level = 2.15059

14

15

Parameter Estimates

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

16

17

Analysis of Deviance Table

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

18

1 AIC: = 40.9353

2

3 **Goodness-of-Fit Table**

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

4

5 Chi² = 3.14 d.f. = 4 *p*-value = 0.5352

6

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

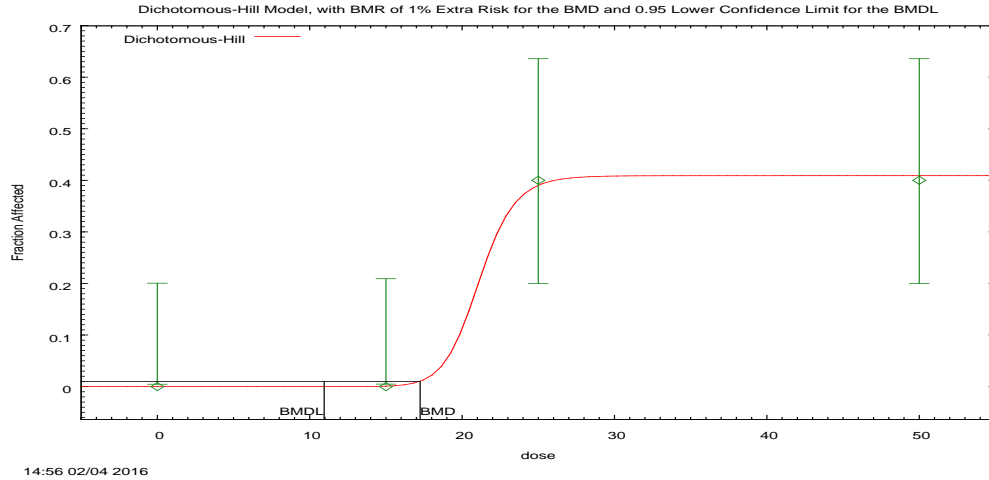
Model ^a	Goodness of fit		BMD _{1Pct} (mg/kg-d)	BMDL _{1Pct} (mg/kg-d)	Basis for model selection
	<i>p</i> -value	AIC			
Gamma	0.0341	66.088	3.14	0.648	All of the models besides dichotomous-Hill had goodness-of-fit <i>p</i> -values <0.10 and thus did not provide an adequate fit to the data. For the dichotomous-Hill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

9

10 ^aThe Multistage 3[°] model may appear equivalent to the Multistage 2[°] model; however, differences exist in digits
 11 not displayed in the table.

12 ^bThe Multistage 2[°] model may appear equivalent to the Multistage 3[°] model; however, differences exist in digits
 13 not displayed in the table.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine



1
2
3
4
5
6
7
8

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

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

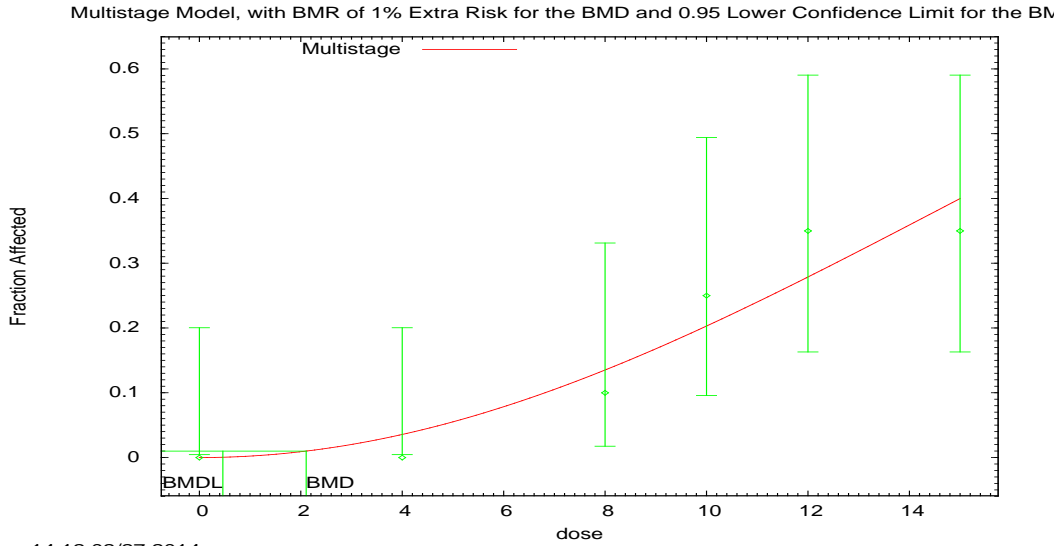
Model ^a	Goodness of fit		BMD _{1Pct} (mg/kg-d)	BMDL _{1Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Gamma	0.794	93.263	3.46	0.840	The multistage 2° model was selected as the representative multistage model based on lowest AIC. From among the multistage 2° and non-multistage models, the multistage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

9
10
11
12
13
14

^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, -0.86, -0.46, 0.53, 0.72, and 0.45, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were 6.82 and 4.41 mg/kg-d, respectively.

^bThe Multistage 5° model may appear equivalent to the Multistage 4° model; however, differences exist in digits not displayed in the table.

1



2

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

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

8 The form of the probability function is: $P[\text{response}] = \text{background} +$
 9 $(1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

10

11 **Benchmark Dose Computation.**

12 BMR = 1% Extra risk

13 BMD = 2.10625

14 BMDL at the 95% confidence level = 0.462994

15

16 **Parameter Estimates**

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

17

1 **Analysis of Deviance Table**

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

2
3 AIC: = 91.926

4
5 **Goodness-of-Fit Table**

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

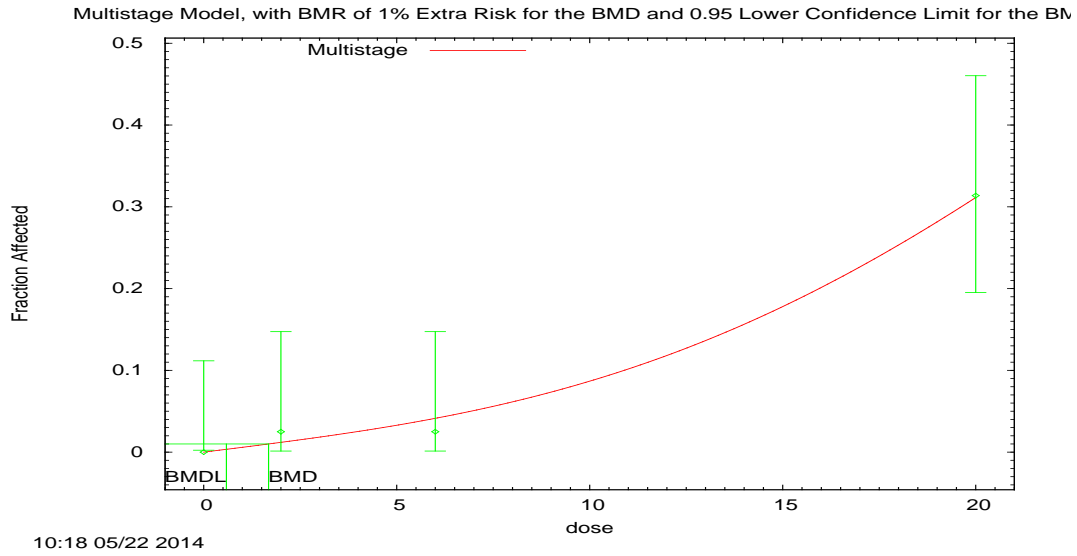
6
7 Chi² = 1.94 d.f. = 5 p-value = 0.8576

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

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

11
12 ^aSelected model in bold; scaled residuals for selected model for doses 0, 2, 6, and 20 mg/kg-day were 0.00, 0.76,
13 -0.52, and 0.04, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were 10.9 and
14 6.09 mg/kg-day, respectively; the BMD₀₅ and BMDL₀₅ estimates for the selected model were 5.23 and
15 7.29 mg/kg-day, respectively.

1



2

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

6

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

8 The form of the probability function is: $P[\text{response}] = \text{background} +$
 9 $(1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

10

11 **Benchmark Dose Computation**

12 BMR = 1% Extra risk

13 BMD = 1.68097

14 BMDL at the 95% confidence level = 0.587568

15

16 **Parameter Estimates**

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

17

1 **Analysis of Deviance Table**

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

2
3 AIC: = 86.9063

4
5 **Goodness-of-Fit Table**

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

6
7 Chi^2 = 0.85 d.f. = 2 p-value = 0.6549

9 **D.2. BENCHMARK DOSE MODELING SUMMARY FOR CANCER ENDPOINTS**

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

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

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

14
15 ^aFor female mouse hepatocellular tumors from [Lish et al. \(1984\)](#), tumor incidence and totals are those reported in
16 the Pathology Working Group (PWG) reevaluation ([Parker et al., 2006](#)).

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

2 First, to determine whether a time-to-tumor analysis was warranted, the survival curves
3 were compared across dose groups for female mice in [Lish et al. \(1984\)](#) in the study to determine
4 whether time of death should be incorporated in the dose-response analysis of tumors. A log-rank
5 test on the Kaplan-Meier survival curves per dose was used to do the comparison, excluding
6 animals that died prior to week 11 when the dose was reduced in the high-dose group to
7 100 mg/kg-day. The test yielded a nonsignificant result ($p = 0.51$), so a time-to-tumor analysis was
8 not necessary for this study.

9 Therefore, non-time-dependent dose-response analyses were conducted using standard
10 BMDS models. For each tumor type, BMDS multistage-cancer models⁶ were fitted to the data using
11 the maximum likelihood method. Each model was tested for goodness-of-fit using a chi-square
12 goodness-of-fit test (χ^2 p -value < 0.05 ⁷ indicates lack of fit). Other factors were used to assess model
13 fit, including scaled residuals, visual fit, and adequacy of fit in the low-dose region and in the
14 vicinity of the BMR. The BMDL estimate and AIC value were used to select a best-fit model from
15 among the models exhibiting adequate fit. If the BMDL estimates were “sufficiently close”
16 (i.e., differed by threefold or less), then the model selected was the one that yielded the lowest AIC
17 value. If the BMDL estimates were not sufficiently close, then the lowest BMDL was selected as the
18 POD.

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

23 **D.2.2. Modeling Results for Mouse Tumor Data**

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

⁶The coefficients of the multistage-cancer models were restricted to be nonnegative (beta values ≥ 0).

⁷A significance level of 0.05 from [U.S. EPA \(2012b\)](#) is used for selecting cancer models because the model family (multistage) is selected a priori.

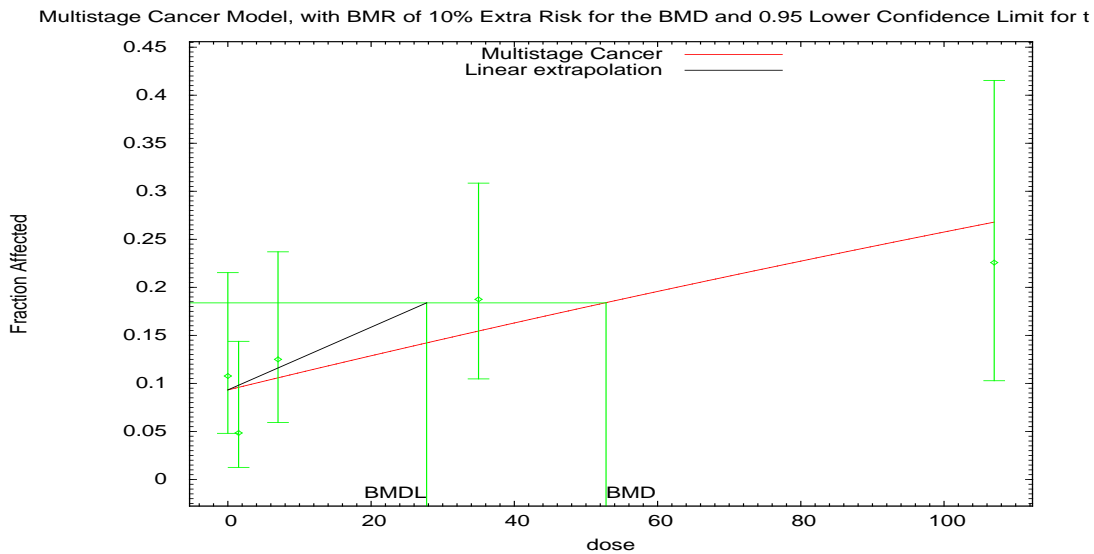
1 **Mouse Tumor Data—BMD Modeling Results**

2 **Table D-16. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months**
 3 **(Lish et al., 1984); BMR = 10% ER**
 4

Model ^a	Goodness of fit		BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^o Multistage 2 ^o Multistage 3 ^o Multistage 4 ^o	0.417	218.68	52.8	27.7	All of the models reduced to the Multistage 1 ^o model, so this model was selected.

5
 6 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were
 7 0.40, -1.27, 0.50, 0.73, and -0.52, respectively.

8 ^bFor the Multistage 2^o, 3^o, and 4^o models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 9 space). The models in this row reduced to the Multistage 1^o model.
 10



11 16:09 02/14 2014
 12 **Figure D-12. Plot of incidence rate by dose, with the fitted curve for the**
 13 **selected model, for combined alveolar/bronchiolar adenoma and carcinoma**
 14 **in female B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al.,**
 15 **1984).**

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

17 The form of the probability function is: $P[\text{response}] = \text{background} +$
 18 $(1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^{1-\text{beta}2} * \text{dose}^2 \dots)]$

19 The parameter betas are restricted to be positive
 20

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Benchmark Dose Computation

BMR = 10% ER

BMD = 52.8078

BMDL at the 95% confidence level = 27.748

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

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

Multistage Cancer Slope Factor = 0.00360387

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 218.682

Goodness-of-Fit Table

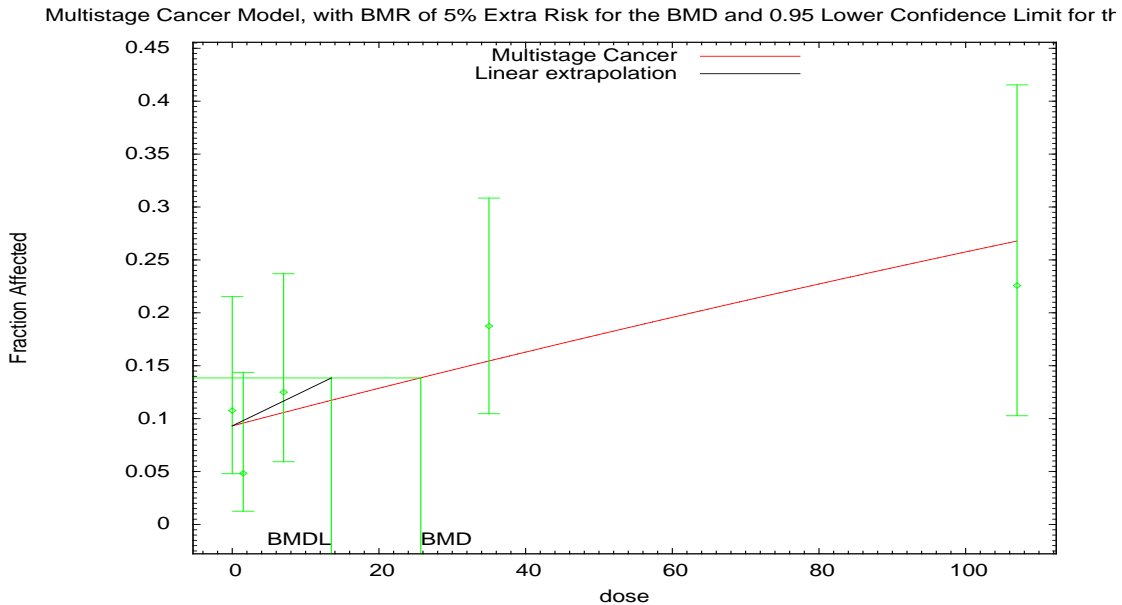
Dose	Est. prob.	Expected	Observed	Size	Scaled residuals
0	0.0932	6.056	7	65	0.403
1.5	0.0959	5.944	3	62	-1.27
7	0.1057	6.768	8	64	0.501
35	0.1543	9.877	12	64	0.734
107	0.2675	8.292	7	31	-0.524

Chi² = 2.84 d.f. = 3 p-value = 0.4168

1 **Table D-17. Model predictions for combined alveolar/bronchiolar adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months**
 2 **(Lish et al., 1984); BMR = 5% ER**
 3

Model ^a	Goodness of fit		BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^{ob}	0.417	218.68	25.7	13.5	All of the models reduced to the Multistage 1 ^o model, so this model was selected.
Multistage 2 ^o					
Multistage 3 ^o					
Multistage 4 ^o					

4
 5 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were
 6 0.40, -0.40, -1.27, 0.50, 0.73, and -0.52, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were
 7 52.8 and 27.7 mg/kg-day, respectively.
 8



10 **Figure D-13. Plot of incidence rate by dose, with fitted curve for selected**
 11 **model, for combined alveolar/bronchiolar adenoma and carcinoma in female**
 12 **B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984).**

13

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 \dots)]$

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 5% ER

BMD = 25.7088

BMDL at the 95% confidence level = 13.5087

BMDU at the 95% confidence level = 94.8384

Taken together, (13.5087, 94.8384) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00370131

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 218.682

Goodness-of-Fit Table

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

Chi² = 2.84 d.f. = 3 p-value = 0.4168

1
2
3

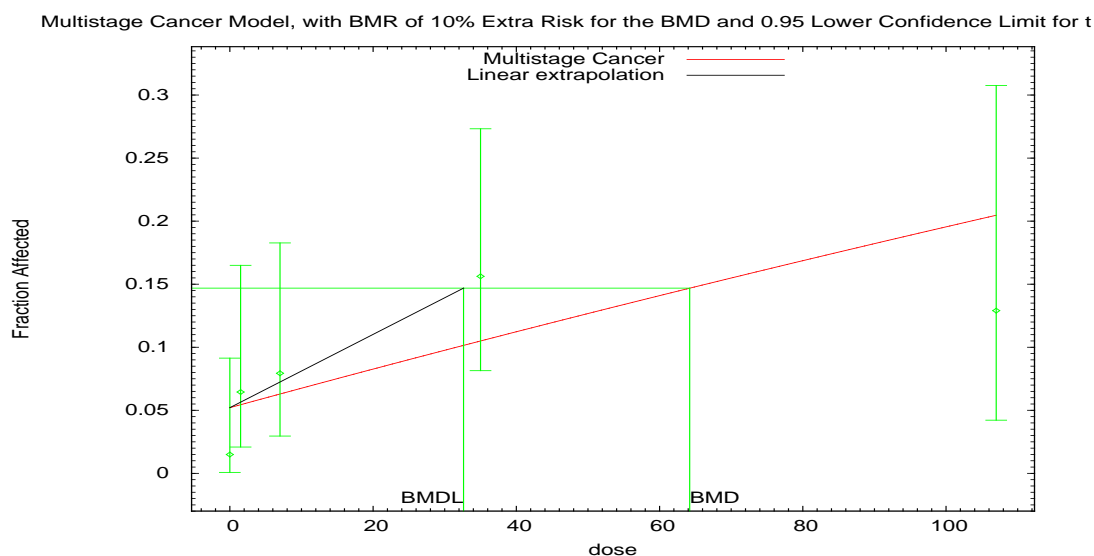
Table D-18. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 10% ER

Model ^a	Goodness of fit		BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^{ob}	0.160	164.06	64.2	32.6	All of the models reduced to the Multistage 1 ^o model, so this model was selected.
Multistage 2 ^o					
Multistage 3 ^o					
Multistage 4 ^o					

4
5
6
7
8
9

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

^bFor the Multistage 2^o, 3^o, and 4^o models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter space). The models in this row reduced to the Multistage 1^o model.



10
11
12
13
14

Figure D-14. Plot of incidence rate by dose, with fitted curve for selected model, for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Parker et al., 2006).

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 \dots)]$

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 10% ER

BMD = 64.203

BMDL at the 95% confidence level = 32.6282

BMDU at the 95% confidence level = 281.385

Taken together, (32.6282, 281.385) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00306483

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 164.063

Goodness-of-Fit Table

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

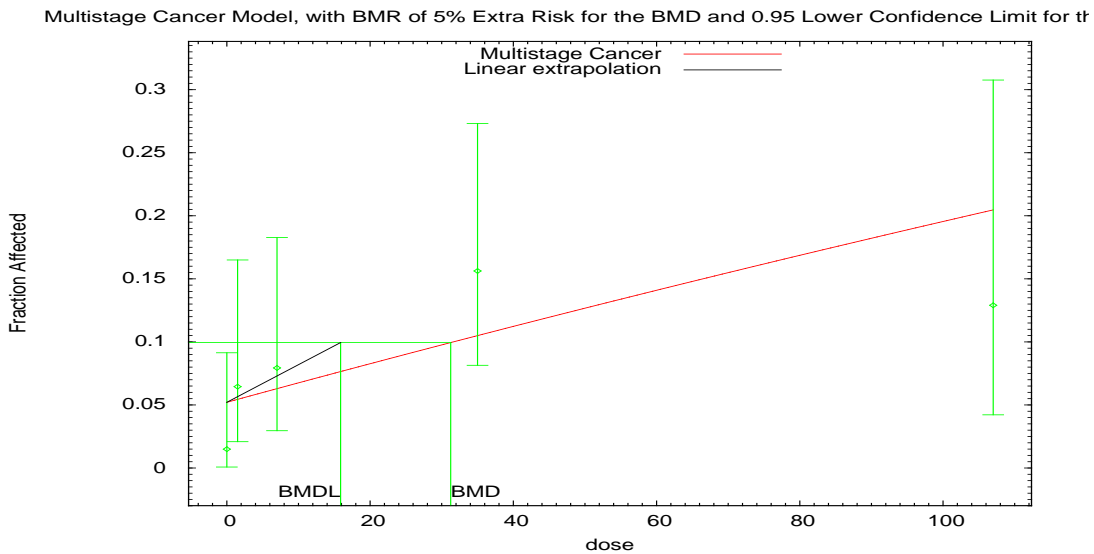
$\chi^2 = 5.17$ d.f. = 3 p-value = 0.16

1 **Table D-19. Model predictions for B6C3F₁ female mouse combined**
 2 **hepatocellular adenoma and carcinoma in mice exposed to RDX by diet for**
 3 **24 months ([Parker et al., 2006](#)); BMR = 5% ER**

Model ^a	Goodness of fit		BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^{ob}	0.160	164.06	31.3	15.9	All of the models reduced to the Multistage 1° model, so this model was selected.
Multistage 2°					
Multistage 3°					
Multistage 4°					

4
 5 ^aSelected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were -1.37,
 6 0.35, 0.54, 1.34, and -1.05, respectively. The BMD₁₀ and BMDL₁₀ values for the selected model were 64.2 and
 7 32.6 mg/kg-day, respectively.

8 ^bFor the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 9 space). The models in this row reduced to the Multistage 1° model.



11
 12 **Figure D-15. Plot of incidence rate by dose, with fitted curve for selected**
 13 **model, for B6C3F₁ female mouse combined hepatocellular adenoma and**
 14 **carcinoma in mice exposed to RDX by diet for 24 months ([Parker et al., 2006](#)).**

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 \dots)]$

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 5% ER

BMD = 31.2563

BMDL at the 95% confidence level = 15.8846

BMDU at the 95% confidence level = 136.989

Taken together, (15.8846, 136.989) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0031477

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 164.063

Goodness-of-Fit Table

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

Chi² = 5.17 d.f. = 3 p-value = 0.16

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 **Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or**
2 **carcinoma in B6C3F₁ female mice exposed to RDX by diet for 24 months; BMR = 10% ER**

3
4 BMD = 29.0 mg/kg-day; BMDL = 17.7 mg/kg-day

5
6 ***MSCOMBO results***

7
8 ***BMR of 10% Extra Risk***

9
10 **** Start of combined BMD and BMDL Calculations.****

11 Combined Log-Likelihood -187.3723596892213

12 Combined Log-likelihood Constant 166.01737626058841

13
14
15
16 Benchmark Dose Computation

17 Specified effect = 0.1

18 Risk Type = Extra risk

19 Confidence level = 0.95

20
21 BMD = 28.9753

22 BMDL = 17.6574

23 Multistage Cancer Slope Factor = 0.00566334

24
25
26
27
28
29
30
31

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 **Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or**
2 **carcinoma in B6C3F₁ female mice exposed to RDX by diet for 24 months; BMR = 5% ER**

3

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

5

6 ***MSCOMBO results***

7

8 ***BMR of 5% Extra Risk***

9

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

11

12 Combined Log-Likelihood -187.3723596892213

13

14 Combined Log-likelihood Constant 166.01737626058841

15

16

17 Benchmark Dose Computation

18

19 Specified effect = 0.05

20

21 Risk Type = Extra risk

22

23 Confidence level = 0.95

24

25 BMD = 14.1062

26

27 BMDL = 8.59627

28

29 Multistage Cancer Slope Factor = 0.00581647

30

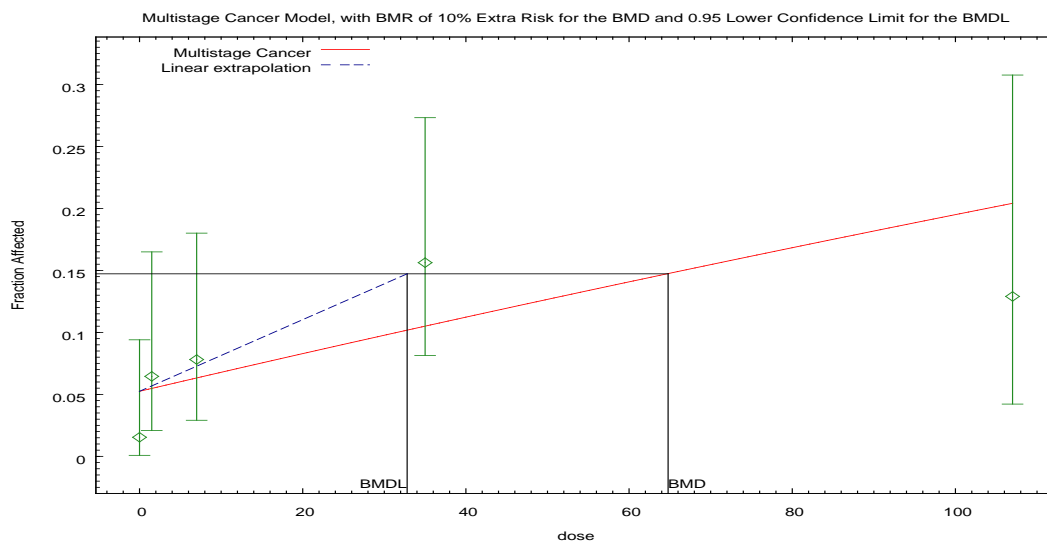
1 **Table D-20. Model predictions for combined hepatocellular adenoma and**
 2 **carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months, using**
 3 **incidence frequencies from [Parker et al. \(2006\)](#) and sample sizes from [Lish et](#)**
 4 **[al. \(1984\)](#); BMR = 10% ER**

Model ^a	Goodness of fit		BMD _{10Pct} (mg/kg-d)	BMDL _{10Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^o Multistage 2 ^o Multistage 3 ^o Multistage 4 ^o	0.171	163.98	64.8	32.8	All of the models reduced to the Multistage 1 ^o model, so this model was selected.

5
 6 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were
 7 -1.34, 0.34, 0.49, 1.34, and -1.03, respectively.

8 ^bFor the Multistage 2^o, 3^o, and 4^o models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 9 space). The models in this row reduced to the Multistage 1^o model.

10



11

12 **Figure D-16. Plot of incidence rate by dose with fitted curve for Multistage-**
 13 **Cancer 1^o model for model predictions for combined hepatocellular adenoma**
 14 **and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months,**
 15 **using incidence frequencies from [Parker et al. \(2006\)](#) and sample sizes from**
 16 **[Lish et al. \(1984\)](#).**

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

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

The form of the probability function is: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 \dots)]$

The parameter betas are restricted to be positive

Benchmark Dose Computation

BMR = 10% Extra risk

BMD = 64.7853

BMDL at the 95% confidence level = 32.7981

BMDU at the 95% confidence level = 291.495

Taken together, (32.7981, 291.495) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00304896

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 163.978

Goodness-of-Fit Table

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

Chi² = 5.02 d.f. = 3 p-value = 0.1706

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 **Combined results for presence of hepatocellular or alveolar/bronchiolar adenoma or**
2 **carcinoma in B6C3F₁ female mice exposed to RDX by diet for 24 months; for hepatocellular**
3 **adenoma or carcinoma, the incidence frequencies from [Parker et al. \(2006\)](#) and the sample**
4 **sizes from [Lish et al. \(1984\)](#) were used; BMR = 10% ER**
5

6 BMD = 29.0 mg/kg-day; BMDL = 17.7 mg/kg-day
7

8 ***MSCOMBO results***
9

10 ***BMR of 10% Extra Risk***
11

12 **** Start of combined BMD and BMDL Calculations.****
13

14 Combined Log-Likelihood -187.33008597565913
15

16 Combined Log-likelihood Constant 166.068416550547
17

18
19 Benchmark Dose Computation
20

21 Specified effect = 0.1
22

23 Risk Type = Extra risk
24

25 Confidence level = 0.95
26

27 BMD = 29.0933
28

29 BMDL = 17.7048
30

31 Multistage Cancer Slope Factor = 0.00564818
32

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

3 This appendix also presents a quantitative dose-response analysis of incidence of liver
 4 carcinomas in male F344 rats ([Levine et al. 1983](#)) and incidence of lung carcinomas in male B3C6F₁
 5 mice (Table D-21). The resulting candidate oral slope factors (OSFs) are presented for comparison
 6 with OSF estimates provided in Section 2.3.3 of the Toxicological Review.

7 **Table D-21. Liver carcinoma data from [Levine et al. \(1983\)](#)**

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

8
 9 ^aThe denominators listed in the table represent the number of animals that were alive 1 year after dosing began.

10
 11 For male mice in [Lish et al. \(1984\)](#), a log-rank test on the Kaplan-Meier survival curves,
 12 stratified by dose, yielded a nonsignificant result (p -value ≥ 0.10), indicating that the survival curves
 13 were similar across dose groups. Therefore, a time-to-tumor analysis was not necessary for
 14 hepatocellular carcinomas in [Lish et al. \(1984\)](#). A non-time-dependent dose-response analysis was
 15 conducted using BMDS multistage-cancer models, and the model selection procedures described in
 16 Section D.2.1 were used to select the appropriate models. Subsequently, the administered dose was
 17 converted to a human equivalent dose (HED) on the basis of (body weight)^{3/4} ([U.S. EPA, 1992](#)), as
 18 described in Section 2.3.2. The POD estimate for male mouse carcinomas and OSF calculated from
 19 this POD are provided in Table D-22; detailed BMD modeling results are provided in Table D-23
 20 (and Figure D-17).

1 **Table D-22. Model predictions and oral slope factor for alveolar/bronchiolar**
 2 **carcinomas in male B6C3F₁ mice exposed to RDX by diet for 2 years ([Lish et al.](#)**
 3 **[1984](#))**

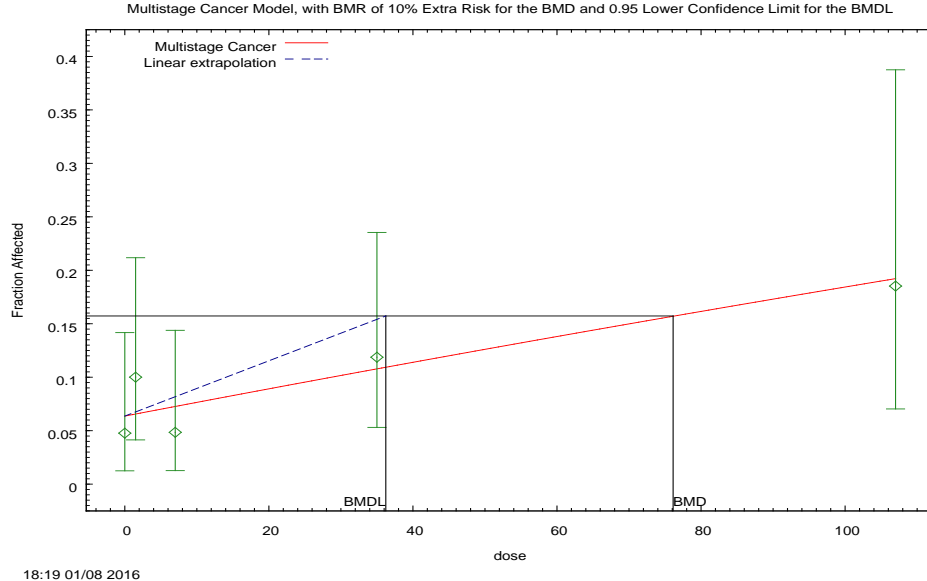
Tumor type	Selected model	BMR	BMD, mg/kg-d	BMDL, mg/kg-d	POD = BMDL _{10-HED} ^a mg/kg-d	Candidate OSF ^b (mg/kg-d) ⁻¹
Alveolar/bronchiolar carcinomas	Multistage 1°	10% ER	76.1	36.2	5.41	0.018

4
 5 ^aBased on allometric scaling of administered RDX dose; BMDL_{10-HED} = BMDL₁₀ × (BW_a^{1/4}/BW_h^{1/4}), BW_a = 0.035 kg,
 6 and BW_h = 70 kg.
 7 ^bSlope factor = BMR/BMDL_{10-HED}, where BMR = 0.10 (10% ER).

8 **Table D-23. Summary of BMD modeling results for model predictions for**
 9 **alveolar/bronchiolar carcinoma in male B6C3F₁ mice exposed to RDX by diet**
 10 **for 2 years ([Lish et al. 1984](#)); BMR = 10% ER**

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

11
 12 ^aSelected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were -0.52,
 13 1.08, -0.74, 0.27, and -0.1, respectively.
 14 ^bFor the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 15 space). The models in this row reduced to the Multistage 1° model.
 16



1

2 **Figure D-17. Plot of incidence rate by dose with fitted curve for Multistage-**
 3 **Cancer 1° model for Model predictions for alveolar/bronchiolar carcinoma in**
 4 **male B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984).**

5

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

7 The form of the probability function is: $P[\text{response}] = \text{background} +$
 8 $(1-\text{background}) * [1-\text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

9 The parameter betas are restricted to be positive

10

11 **Benchmark Dose Computation**

12 BMR = 10% Extra risk

13 BMD = 76.1197

14 BMDL at the 95% confidence level = 36.2316

15 BMDU at the 95% confidence level = 27443100

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

17 Multistage Cancer Slope Factor = 0.00276003

18

19 **Parameter Estimates**

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

20

1 **Analysis of Deviance Table**

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

2
3 AIC: = 162.001

4
5 **Goodness-of-Fit Table**

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

6
7 Chi² = 2.06 d.f. = 3 p-value = 0.561

8
9 For male rats in [Levine et al. \(1983\)](#), the high-dose group had a markedly lower survival
10 curve than the other dose groups, indicating a substantial number of early deaths in the high-dose
11 group. A log-rank test on the Kaplan-Meier survival curves, stratified by dose, yielded a significant
12 result (p-value <0.001), in which case a time-to-tumor analysis is generally preferred. However,
13 such an analysis was not possible because the data were insufficient to allow this analysis.
14 Although tumor incidence was listed for each animal in the source article, the pathology report
15 used a different animal numbering system than the experimental report where the times of death
16 were listed, and the relationship between the two systems was not documented. This prohibited
17 the matching of the times of death and the tumor incidence of the animals, thus prohibiting the use
18 of a time-to-tumor analysis.

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

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

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

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

18 **Table D-24. Model predictions and oral slope factor for hepatocellular**
 19 **carcinomas in male F344 rats administered RDX in the diet for 2 years ([Levine](#)**
 20 **[et al. 1983](#))**

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

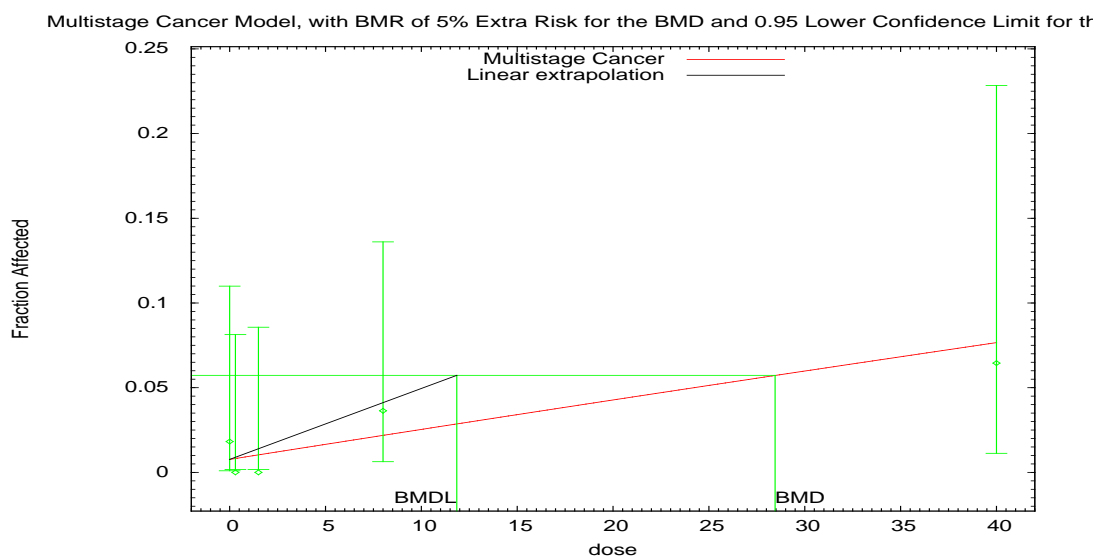
21
 22 ^aSlope factor = BMR/BMDL_{05-HED}, where BMR = 0.05 (5% ER).
 23 ^bBased on allometric scaling of administered RDX dose; BMDL_{05-HED} = BMDL₀₅ × (BW_a^{1/4}/BW_h^{1/4}), BW_a = 0.25 kg, and
 24 BW_h = 70 kg.
 25 ^cBased on toxicological equivalence of PBPK model derived AUC of RDX blood concentration.

1 **Table D-25. Model predictions for combined hepatocellular adenoma and**
 2 **carcinoma in F344 rats exposed to RDX by diet for 24 months ([Levine et al.](#)**
 3 **[1983](#)); BMR = 5% ER**

Model ^a	Goodness of fit		BMD _{5Pct} (mg/kg-d)	BMDL _{5Pct} (mg/kg-d)	Basis for model selection
	p-value	AIC			
Multistage 1^o Multistage 2 ^o Multistage 3 ^o Multistage 4 ^o	0.493	49.095	28.5	11.8	All of the models reduced to the Multistage 1 ^o model, so this model was selected.

4
 5 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were
 6 0.89, -0.67, -0.74, 0.74, and -0.26, respectively.

7 ^bFor the Multistage 2^o, 3^o, and 4^o models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 8 space). The models in this row reduced to the Multistage 1^o model.
 9



10
 11 **Figure D-18. Plot of incidence rate by dose, with fitted curve for selected**
 12 **model, for combined hepatocellular adenoma and carcinoma in F344 rats**
 13 **exposed to RDX by diet for 24 months ([Levine et al.](#), 1983).**

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

15 The form of the probability function is: $P[\text{response}] = \text{background} +$
 16 $(1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

17 The parameter betas are restricted to be positive
 18

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

Benchmark Dose Computation

BMR = 5% ER

BMD = 28.4525

BMDL at the 95% confidence level = 11.8487

BMDU at the 95% confidence level = 235.886

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

Multistage Cancer Slope Factor = 0.00421987

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 49.0947

Goodness-of-Fit Table

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

Chi² = 2.4 d.f. = 3 p-value = 0.493

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

1 The Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was released for
2 a 60-day public comment period on March 10, 2016. Public comments on the assessment were
3 submitted to the U.S. Environmental Protection Agency (EPA) by the U.S. Army Public Health
4 Command and Uniformed Services University of the Health Sciences (posted May 5, 2016), Johns
5 Hopkins Bloomberg School of Public Health, Special Studies in Risk Assessment class (posted May
6 11, 2016), Ronald Melnick (posted May 19, 2016), and an anonymous member of the public (posted
7 May 5, 2016). The anonymous public comment consisted of the word “good,” and is not further
8 discussed in this appendix.

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

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

- 21 • Andy Nong (Health Canada) provided an overview of the physiologically based
22 pharmacokinetic (PBPK) modeling of RDX that provided a framework for the discussion of
23 modeling during the public science meeting. The slides did not provide specific comments
24 on the IRIS assessment.
- 25 • Nancy Beck (American Chemistry Council) raised several broader programmatic topics
26 concerning the Preamble and the use of quantitative analyses for chemicals with suggestive
27 evidence of carcinogenicity. Beck also raised several issues specific to RDX, including
28 further justification for the choice of a benchmark response (BMR) of 1% for convulsions
29 and for selection of a gavage study ([Crouse et al., 2006](#)) over a dietary study as the basis for
30 the reference dose (RfD). Questions related to both issues had been included in the charge
31 to external peer reviewers.

- 1 • Larry Williams (formerly with U.S. Army Public Health Command) presented a slide titled
2 “Realities of Human RDX Dose,” suggesting that if an individual drank water containing RDX
3 (at the chemical’s water solubility), the toxicity of water would be greater than the toxicity
4 of RDX. Williams identified the no-observed-adverse-effect level (NOAEL) in rats from the
5 90-day [Crouse et al. \(2006\)](#) study as 8 mg/kg-day, and estimated the corresponding
6 equivalent dose in humans as 560 mg/kg-day (or approximately 0.5 g). Using a water
7 solubility of RDX of 60 mg/L, Williams estimated that a 70-kg human would need to drink
8 9 L of RDX-saturated water to ingest 0.5 g RDX; he compared this value to a reported LD₅₀
9 for water of 6 L.

10 EPA identified the following issues with this analysis:

- 11 ○ The dose of 8 mg/kg-day in the [Crouse et al. \(2006\)](#) study was not, in fact, a NOAEL, but
12 rather a dose that caused convulsions in 15% of exposed rats and death in 10% of
13 exposed rats.
- 14 ○ Williams’ analysis also failed to consider the statistical uncertainty around a NOAEL. In
15 a study using 10 animals of each sex per dose group as in the [Crouse et al. \(2006\)](#) study,
16 the 95% upper confidence limit on an observed response rate of 0% is 31%.
17 Increasing the sample size to 20 animals per dose group by combining male and female
18 rats would result in an upper confidence limit of 17%. Therefore, in this case, a
19 response greater than 0% at the NOAEL is possible, even in the absence of statistical
20 significance at that dose.
- 21 ○ Williams’ estimation of a human equivalent dose (HED) failed to take into consideration
22 (1) possible differences in susceptibility between rodents and humans, including
23 allometric scaling between rats and humans to account for species differences in
24 toxicokinetics, and (2) potentially greater variation in sensitivity to RDX in the human
25 population than in an in-bred strain of rat.

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

28 **Comments Related to the Mechanisms by which RDX Induces Seizures**

29

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

40

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

10
11 **Comments Related to the Cancer Descriptor**

12
13 **Comment:** Ronald Melnick commented that the cancer descriptor of *suggestive evidence of*
14 *carcinogenic potential* was not supported, and that RDX clearly met the criteria for *likely to be*
15 *carcinogenic to humans* according to the [U.S. EPA \(2005a\)](#) *Guidelines for Carcinogen Risk Assessment*
16 (Cancer Guidelines) because RDX induced dose-related increases in tumors in two species (mouse
17 and rat), in both sexes, and at two sites (liver and lung).

18
19 **EPA Response:** As noted in the Cancer Guidelines ([U.S. EPA, 2005a](#)), “[c]hoosing a descriptor is a
20 matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a
21 wide variety of potential data sets and weights of evidence... Descriptors represent points along a
22 continuum of evidence; consequently, there are gradations and borderline cases that are clarified
23 by the full narrative” (p. 2-51).

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

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

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

7
8 **EPA Response:** Melnick mischaracterized the role of statistical testing in evaluating evidence of an
9 association between exposure and tumor response. In general, trend tests are preferred for
10 evaluating response patterns across dose groups or exposure levels because they are more
11 powerful in detecting overall dose-response trends than multiple pairwise comparisons to control.
12 The presence of statistically significant pairwise comparisons to control does not provide
13 additional information for assessing cancer hazard in this case.

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

22
23 **Comment:** Melnick also offered the following comments on Section 1.3.2:

- 24
- 25 • The incidence of hepatocellular carcinomas in male rats, identified in the Toxicological
26 Review as showing a statistically significant positive trend, should also have been compared
to historical controls because it is a rare tumor in the F344 rat.
 - 27 • It is inappropriate to emphasize the number of tumors in male rats in the mid- and high-
28 dose groups in [Levine et al. \(1983\)](#) without adjusting for differences in the denominators in
29 these groups.
 - 30 • It is misleading to discuss the lack of tumor findings in the [Hart \(1976\)](#) study in Sprague-
31 Dawley rats without discussing the limitations of that study.

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

- 34
- 35 • Text identifying hepatocellular carcinomas in male F344 rats as rare tumors was added to
36 Section 1.3.2. Statistical comparison of the incidence of hepatocellular carcinomas in male
37 rats in the RDX bioassay with historical control data from the National Toxicology Program
had already been presented in Section 1.2.5 of the Toxicological Review.

- 1 • Text on the number of male rats with liver tumors was revised by providing the total
2 number of animals examined. EPA agrees with including the denominator so that the tumor
3 rate can be adjusted for the number of animals examined histopathologically. The incidence
4 had already been provided in Table 1-13.
- 5 • Limitations of the [Hart \(1976\)](#) study had already been discussed in Section 1.2.5 and
6 summarized in Section 1.3.2. Discussion of the limitations of the study in Section 1.3.2 was
7 expanded to include that fact that examination of pathology in treated rats was limited to
8 the high-dose group.

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

11

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

16

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

26

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

37

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

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

17
18 **EPA Response:** The studies by [MacPhail et al. \(1985\)](#), [Cholakis et al. \(1980\)](#), and [Hart \(1976\)](#) were
19 not excluded or determined to be unacceptable studies. Study evaluation was documented in the
20 Literature Search Strategies | Study Selection and Evaluation section; studies determined to be
21 uninformative were identified in that step and were not brought forward into hazard identification.
22 [MacPhail et al. \(1985\)](#), [Cholakis et al. \(1980\)](#), and [Hart \(1976\)](#) were all included in Section 1.2
23 (Hazard Identification) as informative studies, and were considered in weighing the total
24 evidence—positive and negative—for nervous system effects as a human hazard of RDX exposure
25 in Section 1.2.1 (Integration of Nervous System Effects). Unlike the majority of toxicity studies of
26 RDX, these three studies found no evidence of RDX-associated neurotoxicity. Section 1.2.1 provides
27 a discussion of possible reasons that could account for a lack of nervous system response in these
28 studies, but does not dismiss or exclude those findings.

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

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

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

11
12 **Comment:** The public comments stated that justification of the hazard descriptor of *suggestive*
13 *evidence of carcinogenic potential* was considered clear and sufficient. In another section of the
14 comment document, however, the Special Studies in Risk Assessment class noted that it was not
15 immediately clear how EPA applied the Cancer Guidelines, particularly the weight-of-evidence
16 evaluation. Specifically, more detail was requested on specific factors that would increase or
17 decrease weight of evidence, including the number of independent studies with consistent results,
18 multiple observations across species/strain/site, route of administration (including the fact that the
19 most common route of exposure in humans is inhalation but animal studies used oral
20 administration; differences between species), and severity and progression.

21
22 **EPA Response:** Several of the factors identified as contributing to the weight-of-evidence
23 evaluation for carcinogenicity were already considered in Section 1.3.2 (Carcinogenicity). Other
24 considerations, such as route of administration (only bioassays involving dietary exposure were
25 available) did not influence the weight of evidence. As discussed in Section 1.3.2, the descriptor
26 *suggestive evidence of carcinogenic potential* applies to all routes of human exposure, even where
27 there is inadequate testing by an exposure route (i.e., inhalation exposure in the case of RDX), in the
28 absence of convincing evidence to prove otherwise (see Cancer Guidelines, [U.S. EPA, 2005a](#)).

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

32
33 **Comment:** The limitations of gavage dosing studies, as well as why those limitations are of less
34 concern than the limitations from the other studies, should be more clearly stated in Section 2.1.1.

35
36 **EPA Response:** EPA recognizes the influence of method of dosing on the response to RDX in animal
37 toxicity studies. Considerations related to the selection of a gavage study as the basis for the RfD
38 over a dietary study were addressed in Sections 1.2.1 and 2.1.7, and this was identified as a key

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

1 issue in the Executive Summary. In addition, a question regarding the appropriateness of selecting
2 [Crouse et al. \(2006\)](#), which used gavage administration, as the basis for the RfD is included in the
3 charge to external peer reviewers.
4

REFERENCES FOR APPENDICES

- 1
2 [Angerhofer, R; Davis, G; Balezewski, L](#) (1986). Teratological assessment of Trinitro - RDX in rats.
3 (75-51-0573-86). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene
4 Agency.
- 5 [Arfsten, DP; Davenport, R; Schaeffer, DJ](#) (1994). Reversion of bioluminescent bacteria (Mutatox
6 TM) to their luminescent state upon exposure to organic compounds, munitions, and metal
7 salts. *Biomed Environ Sci* 7: 144-149.
- 8 [ATSDR](#) (Agency for Toxic Substances and Disease Registry). (2012). Toxicological profile for RDX
9 (Update) [ATSDR Tox Profile]. (NTIS/10590213). Atlanta, GA: U.S. Department of Health
10 and Human Services, Public Health Service.
- 11 [Bannon, DI](#) (2006). Toxicology study no. 87-XE-059F-06: Biomarkers of RDX exposure in breath of
12 swine, August - September 2006. Aberdeen Proving Ground, MD: U.S. Army Center for
13 Health Promotion and Preventive Medicine.
- 14 [Bannon, DI; Dillman, JF; Hable, MA; Phillips, CS; Perkins, EJ](#) (2009a). Global gene expression in rat
15 brain and liver after oral exposure to the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine
16 (RDX). *Chem Res Toxicol* 22: 620-625. <http://dx.doi.org/10.1021/tx800444k>
- 17 [Bannon, DI; Dillman, JF; Perkins, EJ; Bao, W; Wolfinger, RD; Chu, T; Phillips, CS](#) (2006). Acute RDX
18 exposure and gene expression in the rat brain [Abstract]. Oxford, United Kingdom: Oxford
19 University Press. <http://www.toxicology.org/pubs/docs/Tox/2006Tox.pdf>
- 20 [Bannon, DI; Johnson, M; Williams, L; Adams, V; Perkins, E; Gust, K; Gong, P](#) (2009b). RDX and
21 miRNA expression in B6C3F1 mice [Letter]. *Environ Health Perspect* 117: A98.
22 <http://dx.doi.org/10.1289/ehp.0800276>
- 23 [Barsotti, M; Crotti, G](#) (1949). [Attacchi epileptici come manifestazione di intossicazione
24 professionale da trimetilen-trinitroamina (T4)]. *Med Lav* 40: 107-112.
- 25 [Bhushan, B; Trott, S; Spain, JC; Halasz, A; Paquet, L; Hawari, J](#) (2003). Biotransformation of
26 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a rabbit liver cytochrome P450: Insight
27 into the mechanism of RDX biodegradation by *Rhodococcus* sp. strain DN22. *Appl Environ*
28 *Microbiol* 69: 1347-1351. <http://dx.doi.org/10.1128/AEM.69.3.1347-1351.2003>
- 29 [Brown, RP; Delp, MD; Lindstedt, SL; Rhomberg, LR; Beliles, RP](#) (1997). Physiological parameter
30 values for physiologically based pharmacokinetic models [Review]. *Toxicol Ind Health* 13:
31 407-484. <http://dx.doi.org/10.1177/074823379701300401>
- 32 [Cao, CJ; Reddy, G; Bannon, DI; Johnson, MS](#) (2008). In vitro study of hexahydro-1,3,5-trinitro-1,3,5-
33 triazine (RDX) metabolism in human liver. Aberdeen Proving Ground, MD: U.S. Army Center
34 for Health Promotion and Preventive Medicine.
- 35 [Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III](#) (1980).
36 Mammalian toxicological evaluation of RDX. (DAMD17-78-C-8027). Kansas City, MO:
37 Midwest Research Institute.
- 38 [Cotruvo, JA; Simmon, VF; Spanggord, RJ](#) (1977). Investigation of mutagenic effects of products of
39 ozonation reactions in water. *Ann N Y Acad Sci* 298: 124-140.
40 <http://dx.doi.org/10.1111/j.1749-6632.1977.tb19259.x>
- 41 [Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI](#) (2006). Subchronic oral
42 toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground,
43 MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 [Crouse, LCB; Michie, MW; Major, MA; Leach, GJ; Reddy, G.](#) (2008). Oral bioavailability of
2 cyclotrimethylenetrinitramine (RDX) from contaminated site soils in rats. *Int J Toxicol* 27:
3 317-322. <http://dx.doi.org/10.1080/10915810802366885>
- 4 [Davies, JOJ; Roberts, DM; Hittarage, A; Buckley, NA.](#) (2007). Oral C-4 plastic explosive in humans - A
5 case series. *Clin Toxicol* 45: 454-457. <http://dx.doi.org/10.1080/15563650601118044>
- 6 [Dilley, JV; Tyson, CA; Newell, GW.](#) (1979). Mammalian toxicological evaluation of TNT wastewaters.
7 Volume II. Acute and subacute mammalian toxicity of TNT and the LAP mixture.
8 (ADA080957. DAMD17-76-C-6050.). Menlo Park, CA: SRI International.
9 <http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA080957>
- 10 [Fellows, RJ; Driver, CR; Cataldo, DA; Harvey, SD.](#) (2006). Bioavailability of hexahydro-1,3,5-trinitro-
11 1,3,5-triazine (RDX) to the prairie vole (*Microtus ochrogaster*). *Environ Toxicol Chem* 25:
12 1881-1886. <http://dx.doi.org/10.1897/05-446R1.1>
- 13 [George, SE; Huggins-Clark, G; Brooks, LR.](#) (2001). Use of a *Salmonella* microsuspending bioassay to
14 detect the mutagenicity of munitions compounds at low concentrations. *Mutat Res* 490: 45-
15 56. [http://dx.doi.org/10.1016/S1383-5718\(00\)00150-9](http://dx.doi.org/10.1016/S1383-5718(00)00150-9)
- 16 [Goldberg, DJ; Green, ST; Nathwani, D; McMenamin, J; Hamlet, N; Kennedy, DH.](#) (1992). RDX
17 intoxication causing seizures and a widespread petechial rash mimicking
18 meningococcaemia. *J R Soc Med* 85: 181.
- 19 [Guo, L; Xu, H; Chen, Y; Chang, Y.](#) (1985). Distribution and metabolism of tritium-labeled hexogen in
20 white mice. 3: 335-339.
- 21 [Harrell-Bruder, B; Hutchins, KL.](#) (1995). Seizures caused by ingestion of composition C-4. *Ann*
22 *Emerg Med* 26: 746-748. [http://dx.doi.org/10.1016/S0196-0644\(95\)70049-8](http://dx.doi.org/10.1016/S0196-0644(95)70049-8)
- 23 [Hart, ER.](#) (1974). Subacute toxicity of RDX and TNT in dogs. Final report. (A717530). Kensington,
24 MD: Litton Bionetics, Inc.
25 <https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA035717>
- 26 [Hart, ER.](#) (1976). Two-year chronic toxicity study in rats. (N00014-73-C-0162). Kensington, MD:
27 Litton Bionetics, Inc.
28 <https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA040161>
- 29 [Hathaway, JA; Buck, CR.](#) (1977). Absence of health hazards associated with RDX manufacture and
30 use. *J Occup Med* 19: 269-272.
- 31 [Hess-Ruth, A; Crouse, L; Roszell, L.](#) (2007). RDX pilot development neurotoxicity test in rats.
32 (Toxicology Study No. 85-XC-064Y-07). Aberdeen Proving Ground: U.S. Army Center for
33 Health Promotion and Preventive Medicine.
- 34 [Hett, DA; Fichtner, K.](#) (2002). A plastic explosive by mouth. *J R Soc Med* 95: 251-252.
35 <http://dx.doi.org/10.1258/jrsm.95.5.251>
- 36 [Hollander, AI; Colbach, EM.](#) (1969). Composition C-4 induced seizures: A report of five cases. *Mil*
37 *Med* 134: 1529-1530.
- 38 [Johnson, MS.](#) (2015). Memorandum for National Center for Environmental Assessment (8101R/Dr.
39 Louis D'Amico), U.S. EPA - Office of Research and Development, 1200 Pennsylvania Ave.,
40 NW, Washington, DC 20460. Subject: Additional data from the oral subchronic toxicity of
41 RDX in rats, 2006. (Toxicology study no. 85-XC-5131-03), U.S. Army Center for Health
42 Promotion and Preventative Medicine, Aberdeen Proving Ground, Maryland; Incidence of
43 seizure relative to mortality events. (MCHB-IP-T). Aberdeen Proving Ground, MD:
44 Department of the Army, US Army Institute of Public Health.
- 45 [Kaplan, AS; Berghout, CF; Peczenik, A.](#) (1965). Human intoxication from RDX. *Arch Environ Health*
46 10: 877-883.
- 47 [Kasuske, L; Schofer, JM; Hasegawa, K.](#) (2009). Two marines with generalized seizure activity. *J*
48 *Emerg Nurs* 35: 542-543. <http://dx.doi.org/10.1016/j.jen.2008.05.001>
- 49 [Ketel, WB; Hughes, JR.](#) (1972). Toxic encephalopathy with seizures secondary to ingestion of
50 composition C-4. A clinical and electroencephalographic study. *Neurology* 22: 871-876.

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 [Knepshield, JH; Stone, WJ.](#) (1972). Toxic effects following ingestion of C-4 plastic explosive. In W
2 Keup (Ed.), *Drug Abuse: Current Concepts and Research* (pp. 296-300). Springfield, IL:
3 Charles C. Thomas.
- 4 [Krishnan, K; Crouse, LCB; Bazar, MA; Major, MA; Reddy, G.](#) (2009). Physiologically based
5 pharmacokinetic modeling of cyclotrimethylenetrinitramine in male rats. *J Appl Toxicol* 29:
6 629-637. <http://dx.doi.org/10.1002/jat.1455>
- 7 [Küçükardali, Y; Acar, HV; Özkan, S; Nalbant, S; Yazgan, Y; Atasoyu, EM; Keskin, O; Naz, A; Akyatan, N;
8 Gökben, M; Danaci, M.](#) (2003). Accidental oral poisoning caused by RDX (cyclonite): A report
9 of 5 cases. *J Intensive Care Med* 18: 42-46. <http://dx.doi.org/10.1177/0885066602239123>
- 10 [Lachance, B; Robidoux, PY; Hawari, J; Ampleman, G; Thiboutot, S; Sunahara, GI.](#) (1999). Cytotoxic
11 and genotoxic effects of energetic compounds on bacterial and mammalian cells in vitro.
12 *Mutat Res* 444: 25-39. [http://dx.doi.org/10.1016/S1383-5718\(99\)00073-X](http://dx.doi.org/10.1016/S1383-5718(99)00073-X)
- 13 [Levin, DE; Hollstein, M; Christman, MF; Schwiers, EA; Ames, BN.](#) (1982). A new Salmonella tester
14 strain (TA102) with AT base pairs at the site of mutation detects oxidative mutagens. *Proc*
15 *Natl Acad Sci USA* 79: 7445-7449. <http://dx.doi.org/10.1073/pnas.79.23.7445>
- 16 [Levine, BS; Furedi, EM; Gordon, DE; Barkley, JJ; Lish, PM.](#) (1990). Toxic interactions of the munitions
17 compounds TNT and RDX in F344 rats. *Fundam Appl Toxicol* 15: 373-380.
18 [http://dx.doi.org/10.1016/0272-0590\(90\)90062-O](http://dx.doi.org/10.1016/0272-0590(90)90062-O)
- 19 [Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM.](#) (1981a). Thirteen week oral (diet) toxicity
20 study of trinitrotoluene (TNT), hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine (RDX) and
21 TNT/RDX mixtures in the Fischer 344 rat. Final report. (ADA108447. DAMD17-79-C-9120.
22 DAMD17-79-C-9161). Chicago, IL: IIT Research Institute.
23 <http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA108447>
- 24 [Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM.](#) (1981b). Thirteen week toxicity study of
25 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 rats. *Toxicol Lett* 8: 241-245.
26 [http://dx.doi.org/10.1016/0378-4274\(81\)90108-9](http://dx.doi.org/10.1016/0378-4274(81)90108-9)
- 27 [Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM.](#) (1983). Determination of the chronic
28 mammalian toxicological effects of RDX (twenty-four month chronic
29 toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the
30 Fischer 344 rat): Final report--phase V. Chicago, IL: IIT Research Institute.
- 31 [Lipscomb, JC; Poet, TS.](#) (2008). In vitro measurements of metabolism for application in
32 pharmacokinetic modeling [Review]. *Pharmacol Ther* 118: 82-103.
33 <http://dx.doi.org/10.1016/j.pharmthera.2008.01.006>
- 34 [Lish, PM; Levine, BS; Furedi, EM; Sagartz, JM; Rac, VS.](#) (1984). Determination of the chronic
35 mammalian toxicological effects of RDX: Twenty-four month chronic
36 toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the
37 B6C3F1 hybrid mouse (Volumes1-3). (ADA181766. DAMD17-79-C-9161). Fort Detrick,
38 Frederick, MD: U.S. Army Medical Research and Development Command.
39 <http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA181766>
- 40 [Ma, B; Li, H.](#) (1993). Neurobehavioral effects of hexogen on exposed workers. *Gongye Weisheng yu*
41 *Zhiyebin* 19: 20-23.
- 42 [MacPhail, RC; Walker, QD; Cook, L.](#) (1985). Neurotoxicology of cyclotrimethylenetrinitramine
43 (RDX). Final report. (ADA168266). Research Triangle Park, NC: U.S. Environmental
44 Protection Agency, Health Effects Research Laboratory, Neurotoxicology Division.
45 <https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA168266>
- 46 [Major, MA; Reddy, G; Berge, MA; Patzer, SS; Li, AC; Gohdes, M.](#) (2007). Metabolite profiling of
47 [14C]hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. *J Toxicol*
48 *Environ Health A* 70: 1191-1202. <http://dx.doi.org/10.1080/15287390701252717>

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 [Martin, D; Hart, E.](#) (1974). Subacute toxicity of RDX and TNT in monkeys (pp. 1-216). (ADA044650).
2 Kensington, MD: Litton Bionetics, Inc.
3 <https://ntrl.ntis.gov/NTRL/dashboard/searchResults.xhtml?searchQuery=ADA044650>
- 4 [Merrill, SL.](#) (1968). Ingestion of an explosive material, composition C-4: A report of two cases.
5 USARV Med Bull 40: 5-11.
- 6 [Musick, TJ; Berge, MA; Patzer, SS; Tilch, KR.](#) (2010). Absorption, distribution, metabolism, and
7 excretion of 14C-RDX following oral administration to minipigs (DAAD05-02-P-2319).
8 (ADA526472). Madison, WI: Covance Laboratories Inc. [http://www.dtic.mil/cgi-](http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA526472)
9 [bin/GetTRDoc?AD=ADA526472](http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA526472)
- 10 [Neuwoehner, J; Schofer, A; Erlenkaemper, B; Steinbach, K; Hund-Rinke, K; Eisentraeger, A.](#) (2007).
11 Toxicological characterization of 2,4,6-trinitrotoluene, its transformation products, and two
12 nitramine explosives. Environ Toxicol Chem 26: 1090-1099. [http://dx.doi.org/10.1897/06-](http://dx.doi.org/10.1897/06-471R.1)
13 [471R.1](http://dx.doi.org/10.1897/06-471R.1)
- 14 [NIOSH](#) (National Institute for Occupational Safety and Health). (2012). NIOSH pocket guide to
15 chemical hazards: Cyclonite, CAS 121-82-4. Cincinnati, OH: U.S. Department of Health and
16 Human Services, Centers for Disease Control and Prevention, National Institute for
17 Occupational Safety and Health. <http://www.cdc.gov/niosh/npg/npgd0169.html>
- 18 [OSHA](#) (Occupational Safety & Health Administration). (2012a). Air contaminants. Table Z-
19 Shipyards. CFR 29 CFR 1915, Subpt. P, App A.
- 20 [OSHA](#) (Occupational Safety & Health Administration). (2012b). Gases, vapors, fumes, dusts, and
21 mists. Appendix A to 1926.55-1970 American Conference of Governmental Industrial
22 Hygienists' threshold limit values of airborne contaminants. CFR 29 CFR 1926.55.
- 23 [Özhan, G; Topuz, S; Alpertunga, B.](#) (2003). Determination of cyclonite (RDX) in human plasma by
24 high-performance liquid chromatography. Farmaco 58: 445-448.
25 [http://dx.doi.org/10.1016/S0014-827X\(03\)00069-7](http://dx.doi.org/10.1016/S0014-827X(03)00069-7)
- 26 [Pan, X; Ochoa, KM; San Francisco, MJ; Cox, SB; Dixon, K; Anderson, TA; Cobb, GP.](#) (2013).
27 Absorption, distribution, and biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine
28 (RDX) in B6C3F1 mice (*Mus musculus*). Environ Toxicol Chem 32: 1295-1303.
29 <http://dx.doi.org/10.1002/etc.2188>
- 30 [Pan, X; San Francisco, MJ; Lee, C; Ochoa, KM; Xu, X; Liu, J; Zhang, B; Cox, SB; Cobb, GP.](#) (2007a).
31 Examination of the mutagenicity of RDX and its N-nitroso metabolites using the Salmonella
32 reverse mutation assay. Mutat Res Genet Toxicol Environ Mutagen 629: 64-69.
33 <http://dx.doi.org/10.1016/j.mrgentox.2007.01.006>
- 34 [Pan, X; Zhang, B; Smith, JN; San Francisco, M; Anderson, TA; Cobb, GP.](#) (2007b). N-Nitroso
35 compounds produced in deer mouse (*Peromyscus maniculatus*) GI tracts following
36 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure. Chemosphere 67: 1164-1170.
37 <http://dx.doi.org/10.1016/j.chemosphere.2006.10.077>
- 38 [Paquet, L; Monteil-Rivera, F; Hatzinger, PB; Fuller, ME; Hawari, J.](#) (2011). Analysis of the key
39 intermediates of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) in groundwater: Occurrence,
40 stability and preservation. J Environ Monit 13: 2304-2311.
41 <http://dx.doi.org/10.1039/c1em10329f>
- 42 [Parker, GA; Reddy, G; Major, MA.](#) (2006). Reevaluation of a twenty-four-month chronic
43 toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the
44 B6C3F1 hybrid mouse. Int J Toxicol 25: 373-378.
45 <http://dx.doi.org/10.1080/10915810600846245>
- 46 [Poulin, P; Krishnan, K.](#) (1995). An algorithm for predicting tissue : blood partition coefficients of
47 organic chemicals from n-octanol : water partition coefficient data. J Toxicol Environ Health
48 46: 117-129. <http://dx.doi.org/10.1080/15287399509532021>
- 49 [Poulin, P; Theil, F, -P.](#) (2000). A priori prediction of tissue : plasma partition coefficients of drugs to
50 facilitate the use of physiologically-based pharmacokinetic models in drug discovery. J

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 Pharm Sci 89: 16-35. [http://dx.doi.org/10.1002/\(SICI\)1520-6017\(200001\)89:1<16::AID-](http://dx.doi.org/10.1002/(SICI)1520-6017(200001)89:1<16::AID-)
2 [JPS3>3.0.CO;2-E](http://dx.doi.org/10.1002/(SICI)1520-6017(200001)89:1<16::AID-)
- 3 [Reddy, G; Allen, NA; Major, MA.](#) (2008). Absorption of ¹⁴C-cyclotrimethylenetrinitramine (RDX)
4 from soils through excised human skin. *Toxicol Mech Meth* 18: 575-579.
5 <http://dx.doi.org/10.1080/15376510701703466>
- 6 [Reddy, G; Eisenhut, K; Morrison, SA; Kelly, JA.](#) (1989). Toxicokinetics of ¹⁴C RDX
7 (cyclotrimethylenetrinitramine) in rats after intratracheal administration [Abstract].
- 8 [Reddy, G; Erexson, GL; Cifone, MA; Major, MA; Leach, GJ.](#) (2005). Genotoxicity assessment of
9 hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Int J Toxicol* 24: 427-434.
10 <http://dx.doi.org/10.1080/10915810500366922>
- 11 [Reifenrath, WG; Kammen, HO; Reddy, G; Major, MA; Leach, GJ.](#) (2008). Interaction of hydration,
12 aging, and carbon content of soil on the evaporation and skin bioavailability of munition
13 contaminants. *J Toxicol Environ Health A* 71: 486-494.
14 <http://dx.doi.org/10.1080/15287390801906956>
- 15 [Rispin, A; Farrar, D; Margosches, E; Gupta, K; Stitzel, K; Carr, G; Greene, M; Meyer, W; McCall, D.](#)
16 (2002). Alternative methods for the median lethal dose (LD(50)) test: The up-and-down
17 procedure for acute oral toxicity. *ILAR J* 43: 233-243.
18 <http://dx.doi.org/10.1093/ilar.43.4.233>
- 19 [Safe Work Australia.](#) (2014). Hazardous substances information system (HSIS). Exposure standard
20 documentation: Cyclonite [Database]. Retrieved from
21 [http://www.hsis.safeworkaustralia.gov.au/ExposureStandards/Document?exposureStandards](http://www.hsis.safeworkaustralia.gov.au/ExposureStandards/Document?exposureStandardID=174)
22 [rdID=174](http://www.hsis.safeworkaustralia.gov.au/ExposureStandards/Document?exposureStandardID=174)
- 23 [Schneider, NR; Bradley, SL; Andersen, ME.](#) (1977). Toxicology of cyclotrimethylenetrinitramine:
24 Distribution and metabolism in the rat and the miniature swine. *Toxicol Appl Pharmacol* 39:
25 531-541. [http://dx.doi.org/10.1016/0041-008X\(77\)90144-2](http://dx.doi.org/10.1016/0041-008X(77)90144-2)
- 26 [Schneider, NR; Bradley, SL; Andersen, ME.](#) (1978). The distribution and metabolism of
27 cyclotrimethylenetrinitramine (RDX) in the rat after subchronic administration. *Toxicol*
28 *Appl Pharmacol* 46: 163-171. [http://dx.doi.org/10.1016/0041-008X\(78\)90147-3](http://dx.doi.org/10.1016/0041-008X(78)90147-3)
- 29 [Simmon, VF; Spanggord, RJ; Eckford, SL; McClurg, V.](#) (1977). Mutagenicity of some munition
30 wastewater chemicals and chlorine test kit reagents (pp. 1-205). (ADA057680. DAMD17-
31 76-C-6013). Menlo Park, CA: SRI International.
32 <http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA057680>
- 33 [Smith, JN; Espino, MA; Liu, J; Romero, NA; Cox, SB; Cobb, GP.](#) (2009). Multigenerational effects in
34 deer mice (*Peromyscus maniculatus*) exposed to hexahydro-1,3,5-trinitroso-1,3,5-triazine
35 (TNX). *Chemosphere* 75: 910-914. <http://dx.doi.org/10.1016/j.chemosphere.2009.01.010>
- 36 [Snodgrass, HL, Jr.](#) (1984). Preliminary assessment of relative toxicity and mutagenicity potential of
37 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane (mononitroso-RDX) (Final Report ed.).
38 (ADA149351. USAEHA-75-51-0345-85). Aberdeen Proving Ground, MD: U.S. Army
39 Environmental Hygiene Agency.
40 <http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA149351>
- 41 [Snoeck, V; Huyghebaert, N; Cox, E; Vermeire, A; Saunders, J; Remon, JP; Verschooten, F; Goddeeris,](#)
42 [BM.](#) (2004). Gastrointestinal transit time of nondisintegrating radio-opaque pellets in
43 suckling and recently weaned piglets. *J Control Release* 94: 143-153.
44 <http://dx.doi.org/10.1016/j.jconrel.2003.09.015>
- 45 [Spiteri, NJ.](#) (1982). Circadian patterning of feeding, drinking and activity during diurnal food access
46 in rats. *Physiol Behav* 28: 139-147. [http://dx.doi.org/10.1016/0031-9384\(82\)90115-9](http://dx.doi.org/10.1016/0031-9384(82)90115-9)
- 47 [Stone, WJ; Paletta, TL; Heiman, EM; Bruce, JI; Knepshield, JH.](#) (1969). Toxic effects following
48 ingestion of C-4 plastic explosive. *Arch Intern Med* 124: 726-730.
49 <http://dx.doi.org/10.1001/archinte.1969.00300220078015>

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 [Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS.](#) (2012a). Assessing the
2 non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically
3 based pharmacokinetic (PBPK) modeling [Review]. *Regul Toxicol Pharmacol* 62: 107-114.
4 <http://dx.doi.org/10.1016/j.yrtph.2011.12.007>
- 5 [Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML.](#) (2012b). Cancer mode of action, weight of
6 evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine
7 (RDX). *Regul Toxicol Pharmacol* 64: 205-224.
8 <http://dx.doi.org/10.1016/j.yrtph.2012.07.005>
- 9 [Tan, EL; Ho, CH; Griest, WH; Tyndall, RL.](#) (1992). Mutagenicity of trinitrotoluene and its metabolites
10 formed during composting. *J Toxicol Environ Health A* 36: 165-175.
11 <http://dx.doi.org/10.1080/15287399209531632>
- 12 [Testud, F; Glanclaude, J, -M; Descotes, J.](#) (1996a). Acute hexogen poisoning after occupational
13 exposure. *J Toxicol Clin Toxicol* 34: 109-111.
14 <http://dx.doi.org/10.3109/15563659609020244>
- 15 [Testud, F; Glanclaude, J; Imperatori, J; Le Meur, B; Descotes, J.](#) (1996b). [Acute hexogen poisoning
16 after occupational exposure, report of 2 cases]. *Arch Mal Prof Med Trav Secur Soc* 57: 342-
17 346.
- 18 [Timchalk, C; Nolan, RJ; Mendrala, AL; Dittenber, DA; Brzak, KA; Mattsson, JL.](#) (2002). A
19 physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the
20 organophosphate insecticide chlorpyrifos in rats and humans. *Toxicol Sci* 66: 34-53.
21 <http://dx.doi.org/10.1093/toxsci/66.1.34>
- 22 [U.S. EPA](#) (U.S. Environmental Protection Agency). (1992). A cross-species scaling factor for
23 carcinogen risk assessment based on equivalence of mg/kg^{3/4}/day [EPA Report] (pp.
24 24152-24161). Washington, DC.
- 25 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2005a). Guidelines for carcinogen risk
26 assessment [EPA Report] (pp. 1-166). (EPA/630/P-03/001F). Washington, DC: U.S.
27 Environmental Protection Agency, Risk Assessment Forum.
28 <http://www2.epa.gov/osa/guidelines-carcinogen-risk-assessment>
- 29 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2005b). Supplemental guidance for assessing
30 susceptibility from early-life exposure to carcinogens (pp. 1-125). (EPA/630/R-03/003F).
31 Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum.
- 32 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2012a). Advances in inhalation gas dosimetry for
33 derivation of a reference concentration (RfC) and use in risk assessment (pp. 1-140).
34 (EPA/600/R-12/044). Washington, DC.
35 <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=244650&CFID=50524762&CFTOK>
36 [EN=17139189](https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=244650&CFID=50524762&CFTOKEN=17139189)
- 37 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2012b). Benchmark dose technical guidance (pp.
38 1-99). (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk
39 Assessment Forum. <https://www.epa.gov/risk/benchmark-dose-technical-guidance>
- 40 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2012c). EPA Federal facilities forum issue paper:
41 Site characterization for munitions constituents [EPA Report] (pp. 1-170). (EPA-505-S-11-
42 001). Washington, D.C. [http://www2.epa.gov/fedfac/epa-federal-facilities-forum-issue-](http://www2.epa.gov/fedfac/epa-federal-facilities-forum-issue-paper-site-characterization-munitions-constituents)
43 [paper-site-characterization-munitions-constituents](http://www2.epa.gov/fedfac/epa-federal-facilities-forum-issue-paper-site-characterization-munitions-constituents)
- 44 [U.S. EPA](#) (U.S. Environmental Protection Agency). (2014). RDX PBPK files in acsIX format [EPA
45 Report].
- 46 [von Oettingen, WF; Donahue, DD; Yagoda, H; Monaco, AR; Harris, MR.](#) (1949). Toxicity and potential
47 dangers of cyclotrimethylenetrinitramine (RDX). *J Ind Hyg Toxicol* 31: 21-31.
- 48 [West, RR; Stafford, DA.](#) (1997). Occupational exposures and haematological abnormalities among
49 ordnance factory workers: A case control study. *Leuk Res* 21: 675-680.
50 [http://dx.doi.org/10.1016/S0145-2126\(97\)00042-8](http://dx.doi.org/10.1016/S0145-2126(97)00042-8)

Supplemental Information—Hexahydro-1,3,5-trinitro-1,3,5-triazine

- 1 [Whong, W, -Z; Speciner, ND; Edwards, GS.](#) (1980). Mutagenic activity of tetryl, a nitroaromatic
2 explosive, in three microbial test systems. *Toxicol Lett* 5: 11-17.
3 [http://dx.doi.org/10.1016/0378-4274\(80\)90142-3](http://dx.doi.org/10.1016/0378-4274(80)90142-3)
4 [Williams, LR; Aroniadou-Anderjaska, V; Qashu, F; Finne, H; Pidoplichko, V; Bannon, DI; Braga, MFM.](#)
5 (2011). RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-
6 mediated currents in the amygdala: A mechanism for RDX-induced seizures. *Environ Health*
7 *Perspect* 119: 357-363. <http://dx.doi.org/10.1289/ehp.1002588>
8 [Woody, RC; Kearns, GL; Brewster, MA; Turley, CP; Sharp, GB; Lake, RS.](#) (1986). The neurotoxicity of
9 cyclotrimethylenetrinitramine (RDX) in a child: A clinical and pharmacokinetic evaluation.
10 *Clin Toxicol* 24: 305-319. <http://dx.doi.org/10.3109/15563658608992595>

11