



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

(CASRN 121-82-4)

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

Supplemental Information

March 2016

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

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ABBREVIATIONS

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

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

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., 1983b); 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 January, 2015. The detailed search strategy used to search
3 four of these databases—PubMed, Toxline, Toxcenter, and Toxic Substances Control Act Test
4 Submissions (TSCATS)—is provided in Table B-1. The search strategy used to search the Defense
5 Technical Information Center (DTIC) database is described in Table B-2. The computerized
6 database searches were augmented by review of online regulatory sources, as well as “forward”
7 and “backward” Web of Science searches of two recent reviews (Table B-3). Forward searching
8 was used to identify articles that cited the selected studies (i.e., the two reviews identified in
9 Table B-3), and backward searching was used to identify articles that the selected studies cited.

10 B.1. DEFENSE TECHNICAL INFORMATION CENTER (DTIC) LITERATURE 11 SEARCH AND SCREEN

12 Among the RDX-related citations that were identified in the DTIC database, 826 (722 after
13 duplicate removal within DTIC) were classified with the distribution “Approved for Public Release”,
14 239 (217 after duplicate removal) were classified as “distribution limited to U.S. Government
15 agencies and their Contractors,” and 199 (181 after duplicate removal) were classified as
16 “distribution limited to U.S. Government agencies only.” A preliminary screen of the 1,120 unique
17 citations was performed; 85 citations with unlimited distribution and 10 citations with limited
18 distribution were selected for further review as potential sources of health effects data or
19 supporting information. The remaining 1,025 unlimited and limited-distribution DTIC references
20 not selected for further consideration were not studies of RDX or did not contain information
21 pertinent to the assessment of the health effects of RDX (e.g., documents were related to
22 environmental properties such as leaching, explosive properties, fuel and propellant properties,
23 weapons systems, treatment of wastewater containing explosives, and disposal technologies).

24 The 85 unique citations with unlimited distribution were uploaded to the Health and
25 Environmental Research Online (HERO) website¹ (<http://hero.epa.gov>). The 10 citations with

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.

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1 limited distribution were subject to a more in-depth screen to determine whether the references
2 provided additional primary health effects data and whether the U.S. Environmental Protection
3 Agency (EPA) should seek authorization for public distribution and upload to HERO. A review of
4 the abstract or full-text of the documents associated with the limited-distribution citations resulted
5 in the following determinations:

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

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

20

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 Cyclotrimethylenetrinitramine[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 Cyclotrimethylenetrinitramine[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 |
| Toxline Date: 4/2012 | Notes: Searched CASRN or synonyms; removed invertebrates, aquatic organisms, amphibians, earthworms. | 507 |
| Toxline Date limit: 2011–2/2013 | @OR+("Cyclonite"+"RDX"+"Cyclotrimethylenetrinitramine"+"cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"1,3,5-trinitro-1,3,5-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"+"Cyclotrimethylenetrinitramine"+"Trimethylenetrinitramine"+"Trimethylene+trinitramine"+"Trimethyleentrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"CX+84A"+"Cyklonit"+"Geksogen"+ | 0 |

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

| Database | Terms | Hits |
|---------------------------------------|---|------|
| | "Heksogen"+"Hexogeen"+"Hexolite"+"KHP+281")+@AND+@range+yr+2011+2013+@NOT+@org+pubmed+pubdart+crisp+tscats | |
| Toxline Date limit: 2012–1/2014 | @OR+("Cyclonite"+"RDX"+"Cyclotrimethylenetrinitramine"+"cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"1,3,5-trinitro-1,3,5-triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+@term+@rn+121-82-4)+ @AND+@range+yr+2012+2014+@NOT+@org+pubmed+pubdart+crisp+tscats | 10 |
| | @OR+("1,3,5-Trinitroperhydro-1,3,5-triazine"+"Esaidro-1,3,5-trinitro-1,3,5-triazina"+"Hexahydro-1,3,5-trinitro-1,3,5-triazin"+"Perhydro-1,3,5-trinitro-1,3,5-triazine"+"Cyclotrimethylenenitramine"+"Trimethylenetrinitramine"+"Trimethylene+trinitramine"+"Trimethyleentrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"CX+84A"+"Cyklonit"+"Geksogen"+"Heksogen"+"Hexogeen"+"Hexolite"+"KHP+281")+@AND+@range+yr+2012+2014+@NOT+@org+pubmed+pubdart+crisp+tscats | 0 |
| Toxline Date limit: 2013-1/2015 | @SYN0+@OR+(RDX+"cyclotrimethylene+trinitramine"+"1,3,5-trinitro-1,3,5-triazine"+"1,3,5-Triaza-1,3,5-trinitrocyclohexane"+"1,3,5-Trinitro-1,3,5-triazacyclohexane"+"1,3,5-Trinitrohexahydro-1,3,5-triazine"+"1,3,5-Trinitrohexahydro-s-triazine"+"1,3,5-Trinitroperhydro-1,3,5-triazine"+"CX+84A")+@AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp | 19 |
| | @SYN0+@OR+("Cyclonite"+"Cyclotrimethylenenitramine"+"Cyclotrimethylene trinitramine"+"Hexahydro-1,3,5-trinitro-1,3,5-triazine"+"Hexahydro-1,3,5-trinitro-s-triazine"+"Hexogen"+"Hexolite"+"KHP+281"+"PBX+(af)+108"+"PBXW+108(E)+"Pbx(AF)+108"+"Perhydro-1,3,5-trinitro-1,3,5-triazine")+@AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp | 9 |
| | @SYN0+@OR+("Research+Development+Explosive"+"Royal+Demolition+eXplosive"+"Trimethylenetrinitramine"+"Trinitrocyclotrimethylene+triamine"+"Trinitrotrimethylenetriamine"+"sym-Trimethylene+trinitramine"+@term+@rn+121-82-4+@term+@rn+204655-61-8+@term+@rn+50579-23-2+@term+@rn+53800-53-6+@term+@rn+57608-45-4+@term+@rn+82030-42-0)+ @AND+@range+yr+2012+2015+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats+crisp | 0 |
| TSCATS Date: 2/2013 | @term+@rn+121-82-4+@AND+@org+tscats | 4 |
| TSCATS 2 Date: 1/2015 | 121-82-4 from EPA receipt date 01/01/2000 | 0 |
| TSCATS 8e/FYI Date: 1/2015 | ("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 |
|---|--|---|
| <p>Toxcenter Date: 4/2012</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 ((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> | <p>337 titles screened (20 selected for full records and added to HERO)</p> |
| <p>Toxcenter Date limit: 1/2012– 2/2013</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</p> | <p>26 titles screened (6 selected for full records and added to HERO)</p> |

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

| Database | Terms | Hits |
|--|--|---|
| | <p>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>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 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>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 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>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</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>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</p> <p>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</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>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>Note: Duplicates were removed.</p> | |

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

| Database | Terms | Hits |
|--|---|-------------------------------------|
| DTIC Online Access Controlled Date searched: 1/2015 | <p>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")</p> <p>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")</p> <p>Limited to Content type: Documents</p> | |
| | 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) |

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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 | | |
| <p>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</p> | 3/2013 | 0 citations added |
| <p>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</p> | 3/2013 | 0 citations added |
| <p>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>)</p> | 3/2013 | 0 citations added |
| <p>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>)</p> | 3/2013 | 3 citations added |
| Online regulatory sources | | |
| <p>Combination of Chemical Abstracts Registry Number (CASRN) and synonyms searched on the following websites:</p> | 4/11/2012 | 15 citations added |
| <p>Agency for Toxic Substances and Disease Registry (ATSDR)</p> | 1/27/2014 | 1 citation added |
| <p>http://www.atsdr.cdc.gov/substances/index.asp</p> | 1/22/2015 | 0 citations added |
| <p>(Note: the reference list for the ATSDR toxicological profile for RDX was compared to the search results and relevant references were added)</p> | | |
| <p>California Environmental Protection Agency (Office of Environmental Health Hazard Assessment) (http://www.oehha.ca.gov/risk.html)</p> | | |
| <p>eChemPortal (http://www.echemportal.org/echemportal/participant/page.action?pageID=9)</p> | | |
| <p>EPA Acute Exposure Guideline Levels (http://www.epa.gov/oppt/aegl/pubs/chemlist.htm)</p> | | |
| <p>EPA—IRISTrack/New Assessments and Reviews (http://cfpub.epa.gov/ncea/iristrac/) to find dates (http://www.epa.gov/ncea/iris/index.html) to find data</p> | | |
| <p>EPA National Service Center for Environmental Publications (NSCEP) (http://www.epa.gov/ncepihom/)</p> | | |
| <p>EPA Science Inventory (http://cfpub.epa.gov/si/)</p> | | |
| <p>Federal Docket www.regulations.gov</p> | | |
| <p>Health Canada First Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php)</p> | | |

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

| Selected key reference(s) or sources | Date | Additional references identified |
|---|------|----------------------------------|
| <p>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> | | |

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 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.
 4

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 [¹⁴C]-RDX grown on [¹⁴C]-RDX-amended soils. [¹⁴C]-RDX and plant metabolites of [¹⁴C]-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 CO₂) and 3–5% was retained in the carcass.
17 Feces, urine, and CO₂ 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 CO₂ 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 (50mg/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

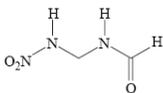
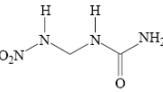
1 [14C]-RDX incorporated plant materials for 5–7 days, with approximately 9% of the administered
2 [14C]-RDX dose eliminated as exhaled [14CO2] ([Fellows et al., 2006](#)).

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 methylenedinitramine
16 and 4-nitro-2-diazbutanal ([Sweeney et al., 2012b](#); [Paquet et al., 2011](#)). RDX metabolism in animals
17 is less well understood. N-Nitroso RDX metabolites have been identified as derived through
18 anaerobic metabolism ([ATSDR, 2012](#); [Pan et al., 2007b](#)). Based on characterization of RDX
19 metabolites in urine and plasma of miniature swine, metabolism of RDX appears to involve loss of
20 nitro groups and ring cleavage ([Musick et al., 2010](#); [Major et al., 2007](#)). The two principal urinary
21 metabolites identified in miniature swine following a single oral dose of 43 or 45 mg/kg were
22 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diaza-butanamide (see Table C-2). [Bhushan et al. \(2003\)](#)
23 suggested that the formation of the 4-nitro-2,4-diazabutanal metabolite occurred via denitration
24 followed by hydroxylation and spontaneous hydrolytic decomposition resulting in ring cleavage
25 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.

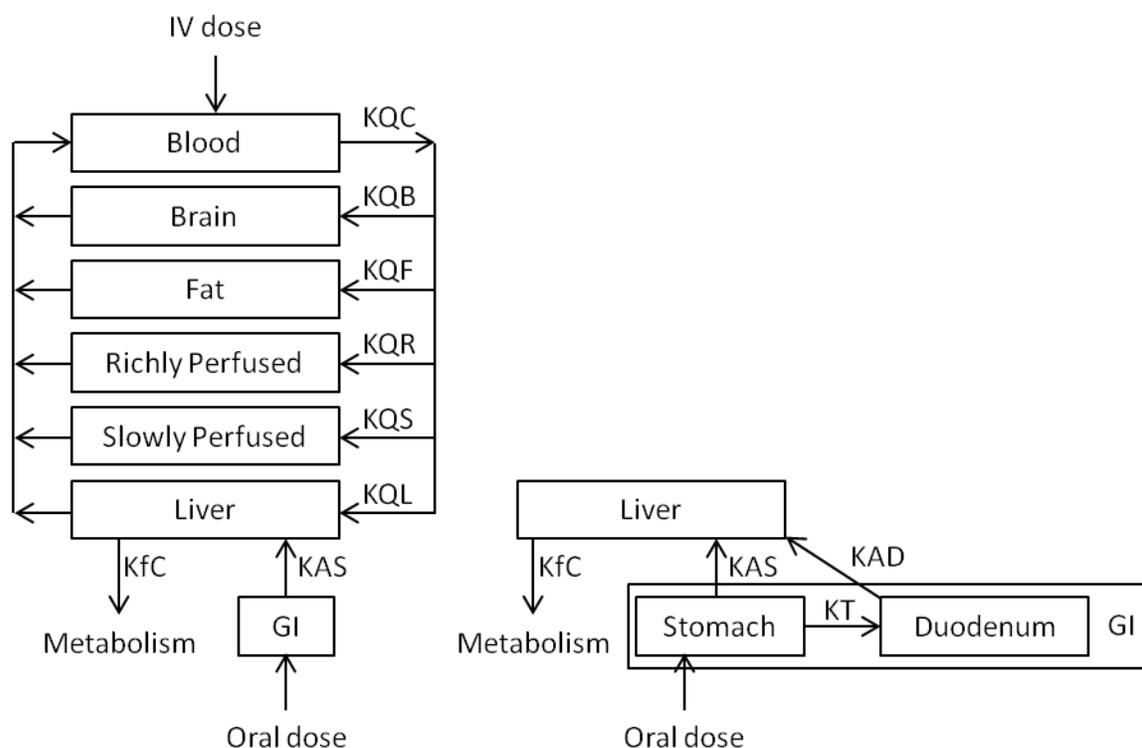
19 ^dCalculated for this review based on reported blood RDX concentrations.

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.**

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**

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

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

29 **Table C-5. Parameters values used in the EPA application of the rat, human,**
 30 **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 | 0.052 | 0.071 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Blood (KVV) | 0.06 | 0.079 | 0.049 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Slowly perfused tissues (KVS) | 0.688 | 0.523 | 0.648 | 0.91 – (KVL + KVB + KVF + KVR + KVV) |
| Blood flows (fraction of cardiac output) | | | | |
| Liver (KQL) | 0.25 | 0.175 | 0.25 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Brain (KQB) | 0.03 | 0.114 | 0.03 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Fat (KQF) | 0.09 | 0.085 | 0.09 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Slowly perfused tissues (KQS) | 0.2 | 0.2449 | 0.2 | Timchalk et al. (2002) ; Brown et al. (1997) |
| Richly perfused tissues (KQR) | 0.43 | 0.3811 | 0.43 | 1 – (KQL + KQB + KQF + KQS) |
| Tissue:blood partition coefficients and metabolism | | | | |
| Liver (PL) | 1.2 | 1.3 | 1.3 | Krishnan et al. (2009)^a |
| Brain (PB) | 1.4 | 1.6 | 1.6 | Krishnan et al. (2009)^a |
| Richly perfused tissues (PR) | 1.4 | 1.6 | 1.6 | Krishnan et al. (2009)^a |
| Fat:blood PC (PF) | 7.55 | 7.55 | 7.55 | Krishnan et al. (2009)^a |
| Slowly perfused tissues (PS) | 1.0 | 1.0 | 0.9 | Krishnan et al. (2009)^a |
| First-order metabolic rate constant (KfC; kg ^{0.33} /hr) | 2.6 | 9.87 (small boy); 11.2 (soldiers) | 77 | Sweeney et al. (2012a)^{b,c} ; Sweeney et al. (2012b)^d |
| Absorption | | | | |
| Absorption from GI to liver (KAS; /hr) | Table C-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 |

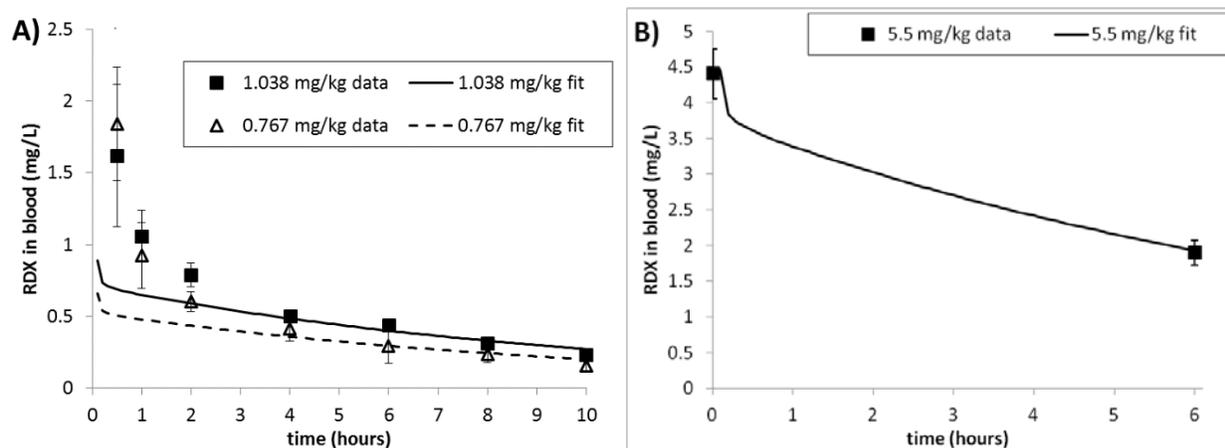
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^aPredicted from n-octanol:water partition coefficient.

^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.

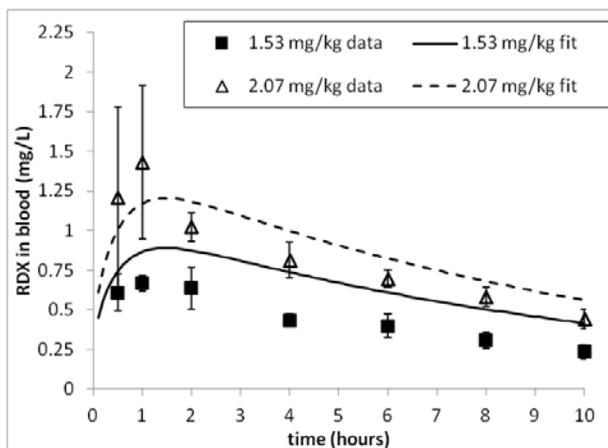
8 **Absorption of RDX from the GI Tract**

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

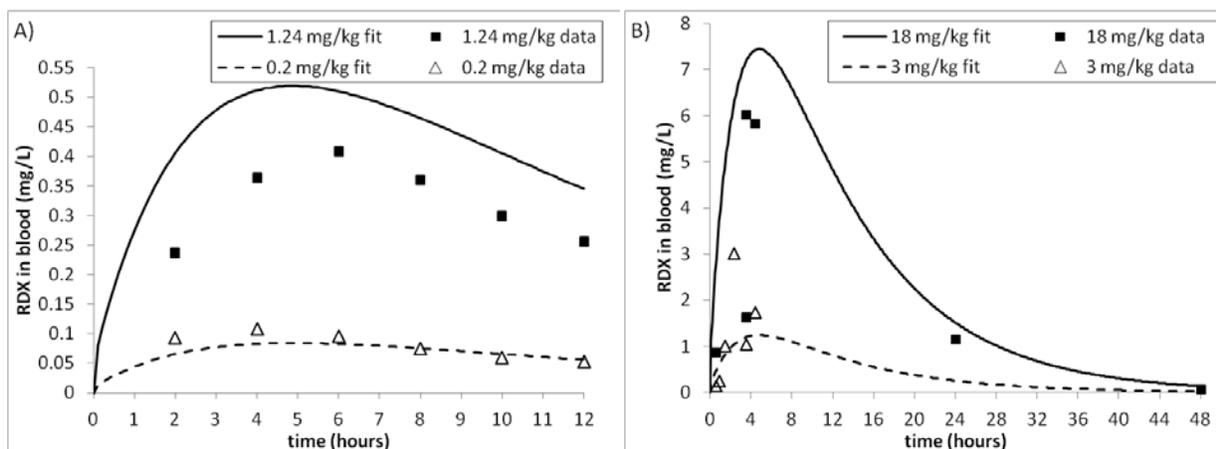


6

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.**

10

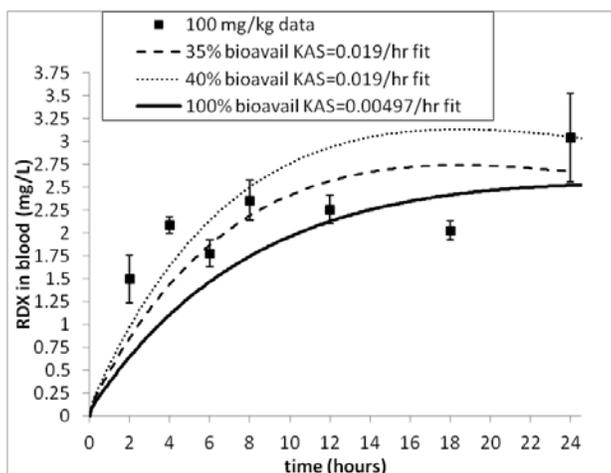


11

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

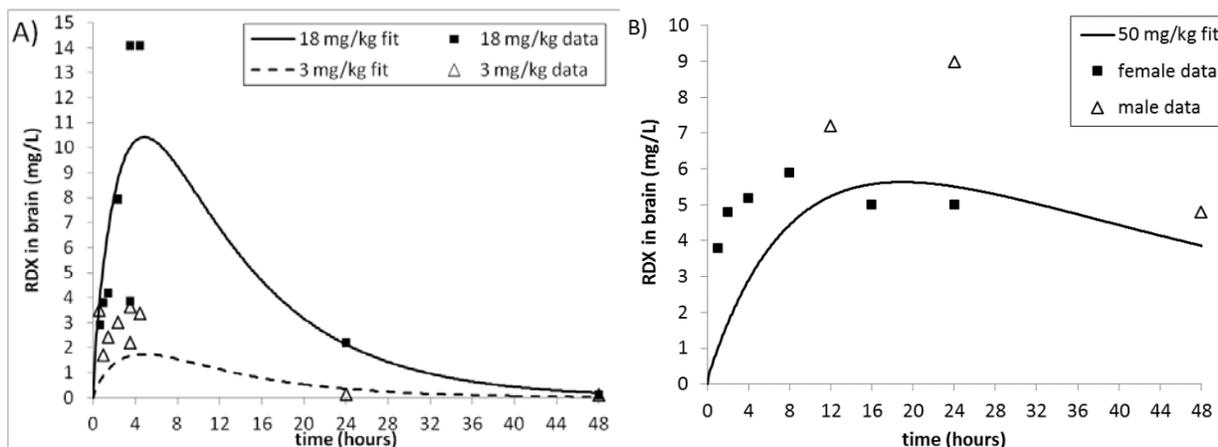
15 **Figure C-4. EPA rat model predictions fitted to observed RDX blood**
 16 **concentrations following oral exposure to RDX in dry capsules.**

17



1
 2 Symbols denote observed RDX blood concentrations measured in male Sprague-Dawley rats
 3 (0.225 kg) resulting from oral doses of 100 mg/kg RDX (Schneider et al., 1977). The KAS fit to
 4 these data assuming 100% bioavailability resulted in the same estimate (KAS = 0.00497/hour) as
 5 obtained by Sweeney et al. (2012a). Alternatively, for KAS fixed at the value fit to fine-grain RDX
 6 in a saline slurry (KAS = 0.019/hour fit to data from Schneider et al. (1977) and MacPhail et al.
 7 (1985); Figure C-7), the estimate of oral bioavailability fit to the RDX blood concentrations was
 8 35%. A bioavailability of 40% and KAS = 0.019/hour is also shown for comparison.
 9

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 A) 3 and 18 mg/kg RDX in dry capsules (0.35 kg male and female rat data from Bannon et al.
 15 (2009a); best fit KAS = 0.35/hour. B) 50 mg/kg fine-grain RDX in a saline slurry (0.25 kg male and
 16 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
2

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

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

3

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

4

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

5

6

7

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

8

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Additional RDX Time-Course Data

12

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

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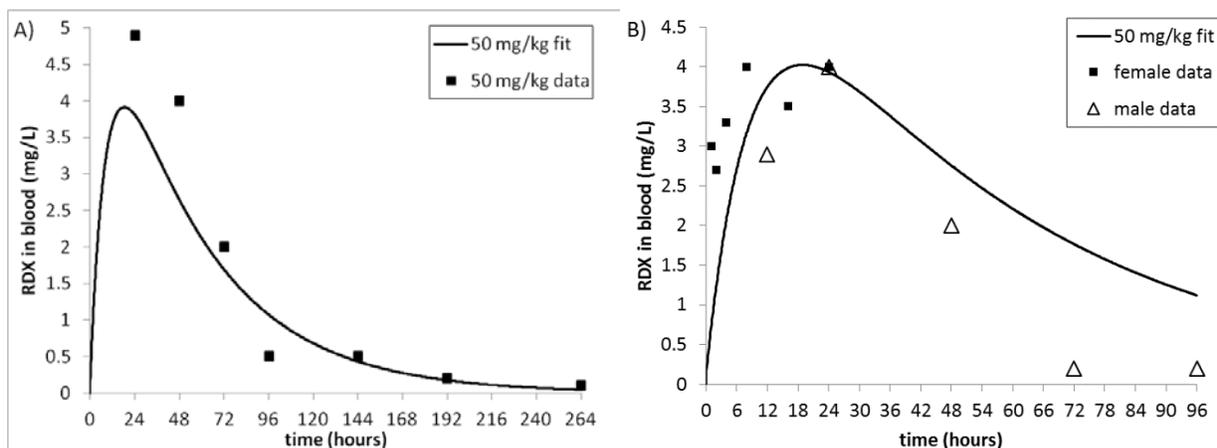
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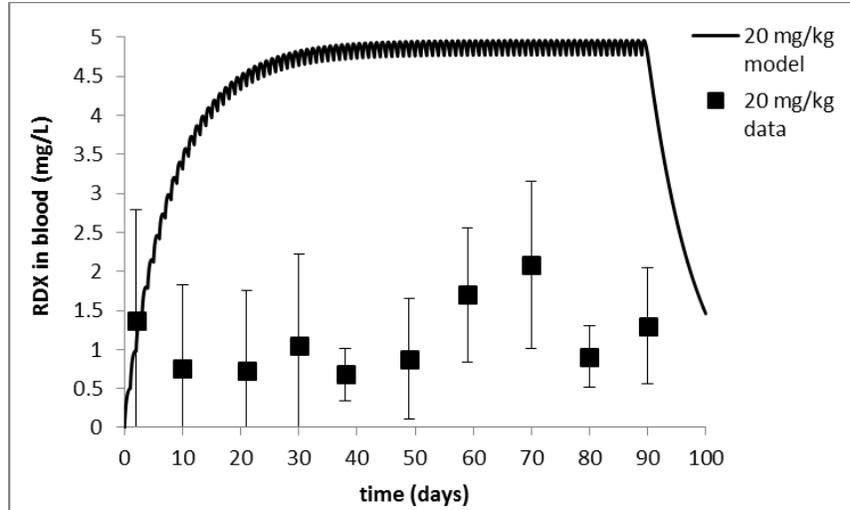
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1
2 Oral doses of 50 mg/kg RDX were administered to: A) male Sprague-Dawley rats (0.225 kg)
3 ([Schneider et al., 1977](#)) and B) male and female Sprague-Dawley rats (0.25 kg) data ([MacPhail et](#)
4 [al., 1985](#)). Best fit KAS = 0.019/hour.

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.



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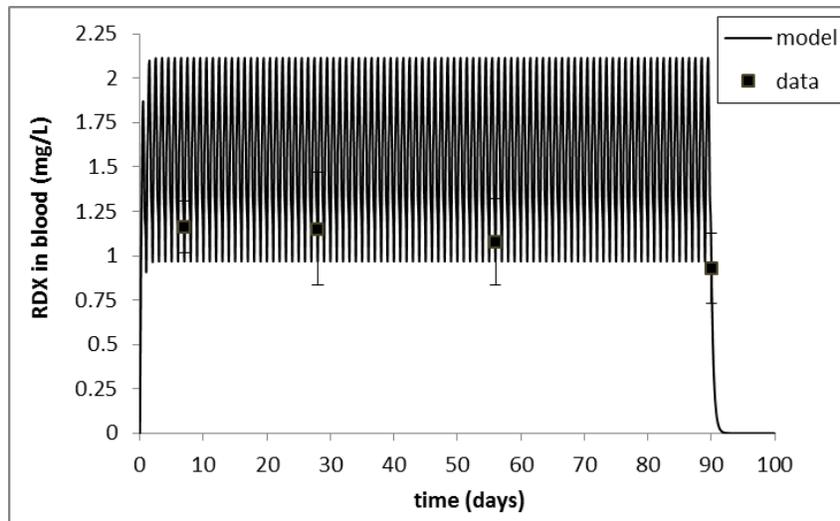
Model fits and mean observed RDX blood concentrations resulting from daily gavage doses of 20 mg/kg RDX for 90 days to male and female Sprague-Dawley rats (0.225 kg). The RDX in saline slurry was assumed to be coarse-grained with an oral absorption rate constant $KAS = 0.00497/\text{hour}$.

6

7

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

8



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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 ($KAS = 1.75/\text{hour}$).

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.

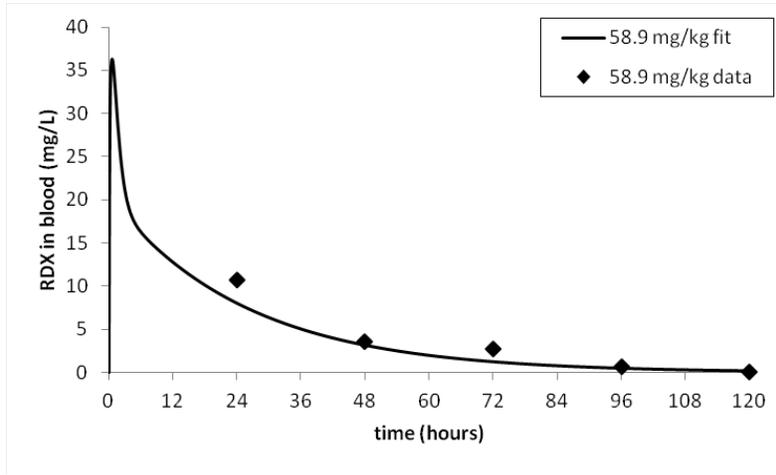
19 It is also possible that metabolic induction occurred during the repeated dosing of RDX in
20 the gavage study leading to the lower observed RDX blood concentrations. The reasonably good fits
21 of the model to the drinking water data set demonstrated achievement of regular periodic levels,
22 and 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

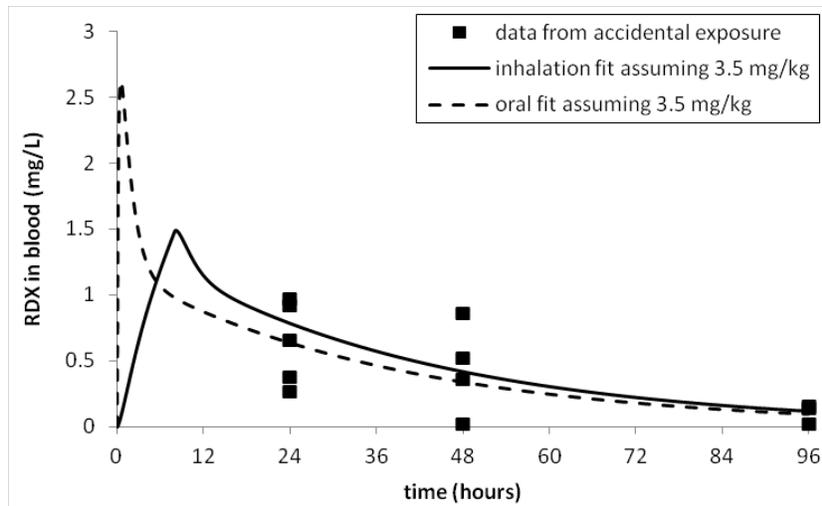
³A constant drinking water ingestion rate interspaced between episodes of no ingestion was assumed. Each 12-hour awake period consisted of eight cycles that alternated between 1.5-hour cycles of frequent sipping (continuous ingestion) and zero ingestion for 45 minutes each. Each 12-hour sleeping period 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 |

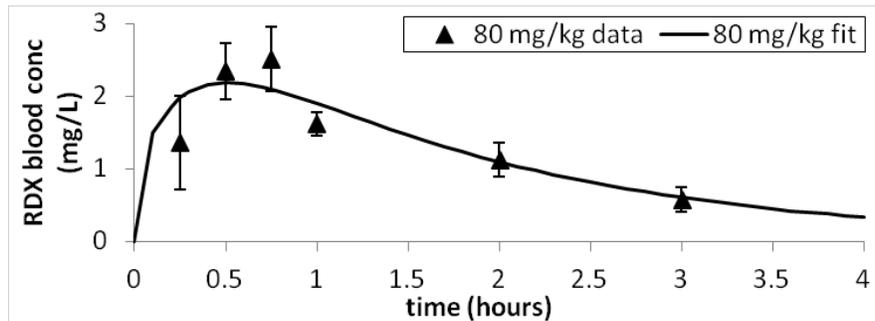
4
 5 Parameters with sensitivity coefficients <0.1 in absolute value are considered not sensitive, and are listed below:
 6 • cardiac output (KQC);
 7 • fractional blood flow to all tissues (liver, KQL; fat, KQF; slowly perfused tissues, KQS; brain, KQB)
 8 • fractional tissue volume of fat (KVF), brain (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.

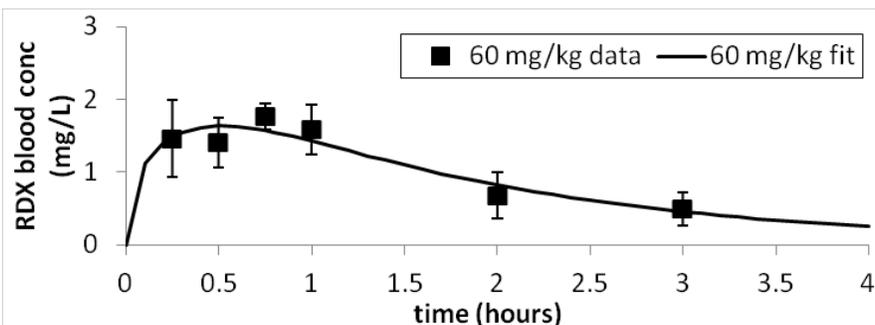
21 ***Simulating Exposures in Mice***

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.

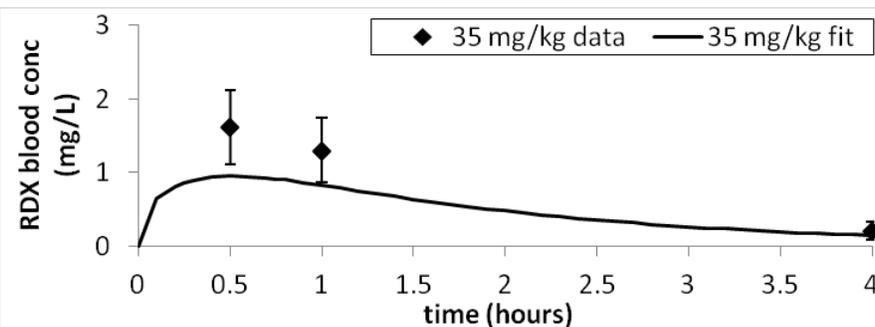
1



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 only information on RDX metabolism in the mouse comes from a study by [Pan et al.](#)
 12 [\(2013\)](#). [Pan et al. \(2013\)](#) measured nitrosamine RDX metabolites of RDX (MNX, DNX, and TNX, the
 13 latter representing a minor metabolic pathway) in mice at the end of a 28-day exposure to RDX in
 14 feed (ad libitum). These measurements were a single time point without controlling the time
 15 between the last RDX ingestion and measurement, and were therefore judged not to be sufficient
 16 for use in parameterizing a PBPK model of the nitrosamine metabolites.

17

1 ***Rat to Human Extrapolations***

2 The rat and human PBPK models as described above were applied to derive human
3 equivalent doses (HEDs) for candidate points of departure (PODs) for endpoints selected from rat
4 bioassays. The rat and human PBPK models were used to estimate two dose metrics—the AUC and
5 the peak concentration (C_{\max}) for RDX concentration in arterial blood. The relationships between
6 administered dose and both internal metrics (AUC and C_{\max}) were evaluated with the rat PBPK
7 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
8 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
9 steady state for the dose metrics were shorter than the duration of the toxicity studies, so the
10 steady state values were considered representative of the study and were used. To calculate
11 steady-state values for daily exposure, the simulations were run until the daily average had a <1%
12 change between consecutive days. For both the rat and human PBPK models, both dose metrics
13 correlated linearly with the administered dose. For rats dosed via gavage, the slope of
14 administered dose versus AUC was 6.800 $\text{mg}/\text{L}\text{-day} / \text{mg}/\text{kg}\text{-day}$ and that for C_{\max} was
15 0.4718 $\text{mg}/\text{L} / \text{mg}/\text{kg}\text{-day}$. For a continuous dose, the slope of dose versus AUC was the same
16 (6.800 $\text{mg}/\text{L}\text{-day} / \text{mg}/\text{kg}\text{-day}$) and for C_{\max} was 0.3951 $\text{mg}/\text{L} / \text{mg}/\text{kg}\text{-day}$. For humans, assuming
17 a drinking water dose sipping pattern, the slope of administered dose versus AUC was
18 13.95 $\text{mg}/\text{L}\text{-day} / \text{mg}/\text{kg}\text{-day}$ and that for C_{\max} was 0.7316 $\text{mg}/\text{L} / \text{mg}/\text{kg}\text{-day}$. Given this linearity
19 in internal metrics and assuming that equal internal metrics in rats and humans are associated with
20 the same degree of response, the HEDs could then be directly determined by multiplying the lower
21 bound on the benchmark dose (BMDL) in rats by the ratio of these slopes. For a gavage dose in rats
22 converted to a human drinking water dose, the ratio for AUC was $6.800 / 13.95 = 0.487$ and C_{\max}
23 was $0.4718 / 0.7316 = 0.645$. For a continuous dose in rats converted to a human drinking water
24 dose, the ratio for AUC was $6.800 / 13.95 = 0.487$ and for C_{\max} was $0.3951 / 0.7316 = 0.540$. These
25 ratios were applied in Table 2-2 to calculate the POD_{HED} from the rat benchmark dose lower
26 confidence limits (BMDLs) and no-observed-adverse-effect levels (NOAELs) for each endpoint.

27 ***Mouse to Human Extrapolations***

28 The mouse and human PBPK models as described above were applied to derive HEDs for
29 candidate PODs for endpoints selected from mouse bioassays. The mouse and human PBPK models
30 were used to estimate two dose metrics—the area under the curve (AUC) and peak concentration
31 (C_{\max}) for RDX concentration in arterial blood. The relationships between administered dose and
32 both internal metrics (AUC and C_{\max}) were evaluated with the mouse PBPK model over the range
33 10 $\mu\text{g}/\text{kg}\text{-day}$ to 100 $\text{mg}/\text{kg}\text{-day}$ and with the human PBPK model over the range 0.05 $\mu\text{g}/\text{kg}\text{-day}$ to
34 200 $\text{mg}/\text{kg}\text{-day}$, ranges that encompass the PODs. The times to reach steady state for the dose
35 metrics were shorter than the duration of the toxicity studies, so the steady state values were
36 considered representative of the study and were used. To calculate steady state values for daily
37 exposure, the simulations were run until the daily average had a <1% change between consecutive

1 days. For both the mouse and human PBPK models, both dose metrics correlated linearly with the
2 administered dose. For mouse dosed via gavage, the slope of administered dose versus AUC was
3 0.0656 mg/L-day / mg/kg-day and that for C_{\max} was 0.0273 mg/L / mg/kg-day. For a continuous
4 dose, the slope of dose versus AUC was the same 0.0656 mg/L-day / mg/kg-day and for C_{\max} was
5 0.0081 mg/L / mg/kg-day. For humans, assuming a drinking water dose sipping pattern, the slope
6 of administered dose versus AUC was 13.95 mg/L-day / mg/kg-day and that for C_{\max} was
7 0.7316 mg/L / mg/kg-day. Given this linearity in internal metrics and assuming that equal internal
8 metrics in mice and humans are associated with the same degree of response, the HEDs could then
9 be directly determined by multiplying the BMDL in mice by the ratio of these slopes. For a gavage
10 dose in mice converted to a human drinking water dose, the ratio for AUC was $0.0656 / 13.95 =$
11 0.0047 and C_{\max} was $0.0273 / 0.7316 = 0.373$, respectively. For a continuous dose in mice
12 converted to a human drinking water dose, the ratio for AUC was $0.0656 / 13.95 = 0.0047$ and for
13 C_{\max} was $0.0081 / 0.7316 = 0.011$. These ratios were applied in Table 2-2 to calculate the POD_{HED}
14 from the mouse BMDLs and NOAELs for each endpoint.

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

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

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

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

- 36 1) RDX is readily metabolized in several species, yet there are no data on the toxicokinetics of
37 RDX metabolites in the rat and human. Some data are available for the n-nitrosoamine

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

- 1 metabolites (a minor metabolic pathway) in mice, but the data are too sparse to help better
2 parameterize a PBPK model. Consequently, the PBPK models used in this assessment do
3 not incorporate the kinetics of RDX metabolites.
- 4 2) The available toxicokinetic data are not sufficient to uniquely identify a parameter value for
5 RDX oral bioavailability. Consequently, the model assumes 100% bioavailability even
6 though some studies in rats suggest that a lower bioavailability is likely.
- 7 3) The human model is based on single accidental exposures, and the exposure concentrations
8 are not known.
- 9 4) The only route of clearance of RDX used in the models is that of total metabolism, which
10 appears reasonable for the rat for which only roughly 5% of the RDX was detected
11 unmetabolized in urine and feces. However, no data on the excretion of RDX are available
12 for the mouse. This inability to properly characterize the fraction of RDX that is
13 metabolized in the mouse is problematic considering some evidence to indicate that the role
14 of metabolism in RDX toxicity may be different across species. This uncertainty decreases
15 the confidence in the mouse PBPK model.
- 16 5) The PBPK model for the mouse is based on a single data set. This single data set is used to
17 fit both the absorption and metabolic rate constants. There are no in vitro data to
18 independently estimate the metabolic rate constant for the mouse. Consequently, the
19 confidence in the mouse model parameter values is low.
- 20 6) The analytical detection limit in the mouse pharmacokinetic study is too high to enable
21 detection at the lower doses. The lowest dose at which RDX was detected was 35 mg/kg;
22 this concentration was high enough to manifest some toxicity in the chronic mouse
23 bioassay. The measured blood concentration at the 35 mg/kg dose is based on the level
24 measured from one single animal (others were non-detects or excluded as outliers). This
25 decreases the confidence in the calibration of the mouse PBPK model.
- 26 7) The metabolic rate constant as estimated by the PBPK model for mice was 30-fold higher
27 than the rat (after accounting for body weight differences), suggesting that the
28 toxicokinetics of RDX could be significantly different in the mouse than in the rat. Mice may
29 have more efficient or higher expression of the CYP450 enzymes. Alternatively, mice may
30 have other unknown metabolic pathways responsible for metabolizing RDX. Identifying the
31 specific CYP450 enzymes and measuring expression levels and in vitro metabolic rate
32 constants in mice would allow for in vitro scaling from rats to mice, which could be used to
33 independently evaluate the mouse metabolic rate constant. Given the high sensitivity of the
34 model to the metabolic rate constant, this uncertainty in the mouse toxicokinetics
35 significantly decreases the confidence in using the mouse PBPK model for predicting mouse
36 blood RDX concentrations.

Model Code for RDX PBPK Model Used in the Assessment

38 The PBPK acslX model code is made available electronically through the Health and
39 Environmental Research Online (HERO) database. All model files may be downloaded in a zipped
40 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 $10.8 \times 10^9/dL$ (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., 1983b](#))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., 1983b](#); [Levine et al., 1981a](#); [Cholakis et al., 1980](#);
19 [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. \(1983b\)](#)), 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., 2006](#))
31 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 Figure 4 in [Levine et al. \(1983a\)](#)); the cause of death in rats that died prior to 6 months on study was generally not determined ([Levine et al., 1983c](#)). 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.,
 5 1983b; Cholakis et al., 1980). In a number of studies, treatment-related mortality was observed at
 6 doses as low as doses associated with nervous system effects (Crouse et al., 2006; Angerhofer et al.,
 7 1986; Levine et al., 1983b; Levine et al., 1981a; Cholakis et al., 1980; von Oettingen et al., 1949).
 8 The 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. (1983b) 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), 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 |

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| 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). | | | | | | |
| Cholakis et al. (1980) | Doses | 0 | 0.2 | 2.0 | 20 | | |
| | Mortality (incidence) | | | | | | |

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

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

- 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 1983b](#)). After 76 weeks of exposure, the incidence of cataracts in female rats at 40 mg/kg-day
14 (23%) was also elevated compared to controls (6%). The incidence of cataracts was not increased
15 in RDX-exposed male rats in the same study ([Levine et al., 1983b](#)), and other studies have not
16 observed ocular effects associated with RDX exposure. Only two rats (dose groups not reported)
17 were observed to have mild cataracts in a 90-day study of male and female F344 rats exposed to
18 RDX at doses up to 15 mg/kg-day by gavage; however, the authors noted that these observations
19 are common in F344 rats at 4 months of age and should not be attributed to treatment ([Crouse et
20 al., 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., 1983b](#)); 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., 1983b](#); [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., 1983b](#)) 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., 1983b](#); [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., 1983b](#); [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., 1983b](#); [Cholakis 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., 1983b](#); [Cholakis 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) | | | |
| | Test | Referent (n = 237) | RDX exposed* 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) | | | |
| | Test (abnormal range) | Referent | RDX exposed* 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. (1983b) 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. (1983b) 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% | 0% | –2% | 6% | |
| | F | 0% | 0% | 0% | –2% | 2% | |
| [#] Includes one affected and three unaffected animals that died prematurely. [†] Includes one unaffected animal that died prematurely. [‡] Minimal severity in four rats, moderate in one. | | | | | | | |
| <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), 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. (1983b) 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 | | | | | | |
|---|---|----|------|------|------|------|-------|
| <p>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</p> <p>Diet 13 wks</p> | 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% | | |
| <p>Cholakis et al. (1980)</p> <p>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</p> <p>Diet 13 wks</p> | 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% |
| <p>Cholakis et al. (1980)</p> <p>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</p> <p>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</p> | 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), 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. (1983b) 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 | <table border="1"> <thead> <tr> <th>Doses</th> <th>0</th> <th>1.5</th> <th>7.0</th> <th>35</th> <th>175/100</th> </tr> </thead> <tbody> <tr> <td colspan="6">RBC count; 105 wks (percent change compared to control)</td> </tr> <tr> <td>M</td> <td>0%</td> <td>-4%</td> <td>3%</td> <td>-3%</td> <td>14%</td> </tr> <tr> <td>F</td> <td>0%</td> <td>4%</td> <td>-7%</td> <td>5%</td> <td>3%</td> </tr> <tr> <td colspan="6">Hemoglobin; 105 wks (percent change compared to control)</td> </tr> <tr> <td>M</td> <td>0%</td> <td>-6%</td> <td>3%</td> <td>-5%</td> <td>9%</td> </tr> <tr> <td>F</td> <td>0%</td> <td>2%</td> <td>-7%</td> <td>3%</td> <td>1%</td> </tr> <tr> <td colspan="6">Hematocrit; 105 wks (percent change compared to control)</td> </tr> <tr> <td>M</td> <td>0%</td> <td>-4%</td> <td>3%</td> <td>-4%</td> <td>9%</td> </tr> <tr> <td>F</td> <td>0%</td> <td>3%</td> <td>-6%</td> <td>3%</td> <td>1%</td> </tr> <tr> <td colspan="6">Platelets; 105 wks (percent change compared to control)</td> </tr> <tr> <td>M</td> <td>0%</td> <td>33%</td> <td>9%</td> <td>21%</td> <td>27%</td> </tr> </tbody> </table> | 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% |
| | 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. (1983b) 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 |
| 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), 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% |
| Martin and Hart (1974) Monkeys, Cynomolgus or Rhesus ^c , 3/sex/group Purity of test material not specified 0, 0.1, 1, or 10 mg/kg-d Gavage 13 wks | Histopathological examination revealed increased numbers of degenerate or necrotic megakaryocytes in all bone marrow sections. | | | | |
| | Doses | 0 | 0.1 | 1 | 10 |
| | RBC count (percent change compared to control) | | | | |
| | M | 0% | -3% | 2% | -3% |
| | F | 0% | 0% | -1% | 2% |
| | Reticulocyte count (percent change compared to control) | | | | |
| | M | 0% | -33% | -50% | -50% |
| | F | 0% | -18% | -36% | 45% |
| | Hematocrit (percent change compared to control) | | | | |
| | M | 0% | -7% | -4% | -1% |
| | F | 0% | 10% | 7% | 3% |
| | Hemoglobin (percent change compared to control) | | | | |
| | M | 0% | -10% | -8% | -6% |
| | F | 0% | 6% | 6% | 3% |

- 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](#); [Minor et al., 1982](#); [Cholakis et al., 1980](#); [Whong et al.,
20 1980](#); [Simmon et al., 1977b](#)). 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 ([Simmon et al., 1977b](#)). The study authors noted
26 that the negative findings should be considered in the context of the low concentrations tested.
27 Similarly, RDX did not induce forward mutations (HGPRT locus) in V79 Chinese hamster lung cells,
28 with or without metabolic activation, although minimal cytotoxicity was observed at 180 μM
29 ([Lachance et al., 1999](#)). However, RDX produced revertants in two of three trials in the Mutatox
30 assay with the bacterium *Vibrio fischeri* when tested at doses up to 2.5 $\mu\text{g}/\text{tube}$, with and without S9
31 ([Arfsten et al., 1994](#)). In the presence of S9, a dose-response was observed; in the absence of S9, no
32 dose-response relationship was detected ([Arfsten et al., 1994](#)). RDX did not induce forward
33 mutations in mouse lymphoma L5178Y cells with or without metabolic activation ([Reddy et al.,
34 2005](#)). During an accompanying range-finding study, precipitates occurred at doses ≥ 250 $\mu\text{g}/\text{mL}$,
35 suggesting that concentrations of RDX in DMSO 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. (1977a) |
| 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>Vibrio fischeri</i> | 0.004 µg/tube | ± | + | Mutatox assay with metabolic activation (S9) | Arfsten et al. (1994) |
| 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) |

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 (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. (1977a) |
| 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 ^aLowest effective dose for positive results; highest dose tested for negative results.
3 ^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](#)).

34

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> TA1535, TA1537, TA1538, TA98, TA100 | 500 µg/plate | + | + | Positive 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. (1983b) | 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%) |

| 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 | 3/20 (15%) |
| | | 10 | 6/20 (30%) |
| | | 12 | 13/20 (65%) |
| | | 15 | 12/20 (60%) |

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

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

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

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

23 The BMDL estimate (95% lower confidence limit on the benchmark dose [BMD]), as
24 estimated by the profile likelihood method) and Akaike’s information criterion (AIC) value were

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 used to select a best-fit model from among the models exhibiting adequate fit. If the BMDL
 2 estimates were “sufficiently close” (i.e., differed by at most threefold), the model selected was the
 3 one that yielded the lowest AIC value. If the BMDL estimates were not sufficiently close, the lowest
 4 BMDL was selected as the POD.

5 **D.1.2. Modeling Results**

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

8 ***Nervous System Effects***

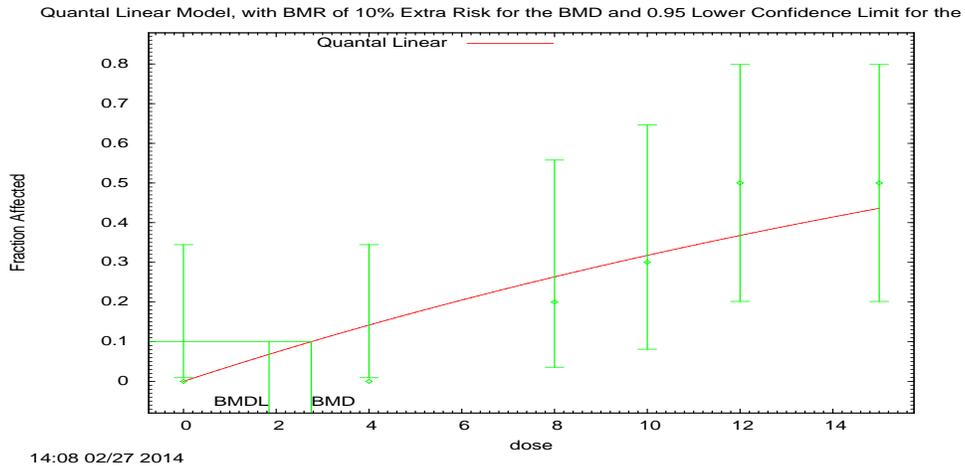
9 Tables D-3 to D-11 (and Figures D-1 to D-9) present the BMD modeling results for incidence
 10 of convulsions for female, male, and male and female F344 rats combined based on data from
 11 [Crouse et al. \(2006\)](#), using BMRs of 10, 5, and 1% extra risk (ER). Tables D-12 to D-14 (and Figures
 12 D-10 to D-12) present the BMD modeling results for incidence of convulsions for female F344 rats
 13 based on data from [Cholakakis et al. \(1980\)](#), using BMRs of 10, 5, and 1% ER. Table D-15 (and Figure
 14 D-13) presents the BMD modeling results for combined incidence of convulsions and mortality for
 15 male and female rats combined based on data from [Crouse et al. \(2006\)](#).

16 **Table D-3. Model predictions for convulsions in female F344 rats exposed to**
 17 **RDX by gavage for 90 days ([Crouse 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 | | | |
| Gamma | 0.923 | 55.085 | 6.46 | 2.92 | The Quantal-Linear model was selected based on lowest BMDL (BMDLs differed by more than threefold). |
| Logistic | 0.733 | 56.607 | 6.76 | 4.75 | |
| LogLogistic | 0.929 | 55.076 | 6.42 | 3.04 | |
| Probit | 0.793 | 56.086 | 6.64 | 4.54 | |
| LogProbit | 0.952 | 54.798 | 6.54 | 3.39 | |
| Weibull | 0.892 | 55.420 | 6.16 | 2.62 | |
| Multistage 2° | 0.954 | 53.595 | 5.46 | 2.47 | |
| Quantal-Linear | 0.733 | 56.131 | 2.76 | 1.84 | |
| Multistage 5 ^{ob} | 0.885 | 55.525 | 5.98 | 2.47 | |
| Multistage 4° | 0.885 | 55.525 | 5.98 | 2.47 | |
| Multistage 3° | 0.885 | 55.525 | 5.98 | 2.49 | |
| Dichotomous-Hill | 0.964 | 56.265 | 7.10 | 5.75 | |

18
 19 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 20 -1.29, -0.46, -0.12, 0.87, and 0.41, respectively.

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



4
 5 BMR = 10% ER; dose shown in mg/kg-day.

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

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

10 The form of the probability function is: $P[\text{response}] = \text{background} +$
 11 $(1-\text{background}) * [1 - \text{EXP}(-\text{slope} * \text{dose})]$

12
 13 **Benchmark Dose Computation**

14 BMR = 10% ER
 15 BMD = 2.75544
 16 BMDL at the 95% confidence level = 1.84489

17
 18 **Parameter Estimates**

| Variable | Estimate | Default initial parameter values |
|------------|----------------|----------------------------------|
| Background | 0 | 0.0833333 |
| Slope | 0.0382372 | 0.0404091 |
| Power | Not applicable | 1 |

19
 20 **Analysis of Deviance Table**

| Model | Log (likelihood) | Number of parameters | Deviance | Test degrees of freedom (d.f.) | p-value |
|------------|------------------|----------------------|----------|--------------------------------|---------|
| Full model | -24.9756 | 6 | | | |

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

| | | | | | |
|---------------|----------|---|---------|---|----------|
| Fitted model | -27.0654 | 1 | 4.17949 | 5 | 0.5239 |
| Reduced model | -33.7401 | 1 | 17.529 | 5 | 0.003598 |

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AIC: = 56.1307

Goodness-of-Fit Table

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

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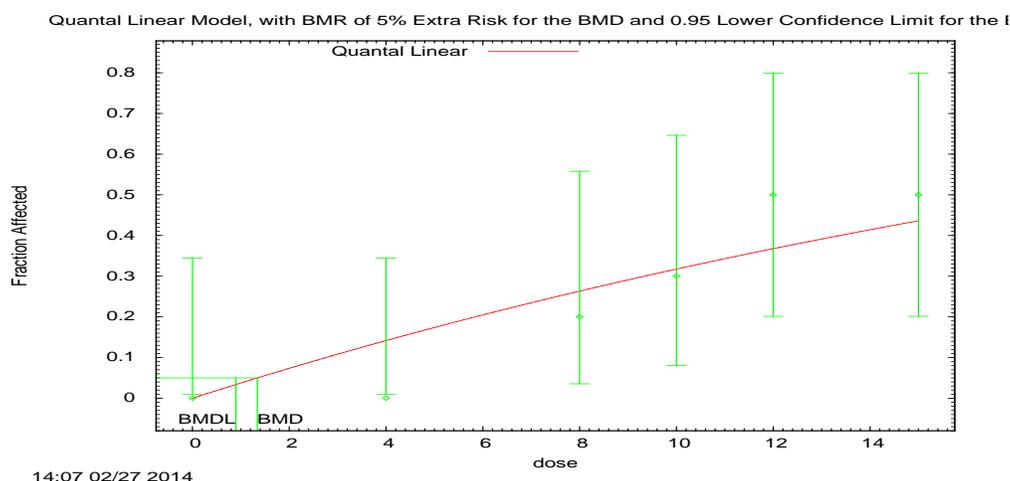
^aEstimated probability

Chi² = 2.79 d.f. = 5 *p*-value = 0.7325

1 **Table D-4. Model predictions for convulsions in female F344 rats exposed to**
 2 **RDX by gavage for 90 days (Crouse et al., 2006); BMR= 5% ER**

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

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 5 -1.29, -0.46, -0.12, 0.87, and 0.41, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 2.76 and
 6 1.84 mg/kg-day, respectively.
 7 ^bThe Multistage 4° model may appear equivalent to the Multistage 3° model; however, differences exist in digits
 8 not displayed in the table.



9
 10 BMR = 5% ER; dose shown in mg/kg-day.

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

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

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

Benchmark Dose Computation

BMR = 5% ER

BMD = 1.34145

BMDL at the 95% confidence level = 0.89816

Parameter Estimates

| Variable | Estimate | Default initial parameter values |
|------------|----------------|----------------------------------|
| Background | 0 | 0.0833333 |
| Slope | 0.0382372 | 0.0404091 |
| Power | Not applicable | 1 |

Analysis of Deviance Table

| Model | Log (likelihood) | Number of parameters | Deviance | Test d.f. | p-value |
|---------------|------------------|----------------------|----------|-----------|----------|
| Full model | -24.9756 | 6 | | | |
| Fitted model | -27.0654 | 1 | 4.17949 | 5 | 0.5239 |
| Reduced model | -33.7401 | 1 | 17.529 | 5 | 0.003598 |

AIC: = 56.1307

Goodness-of-Fit Table

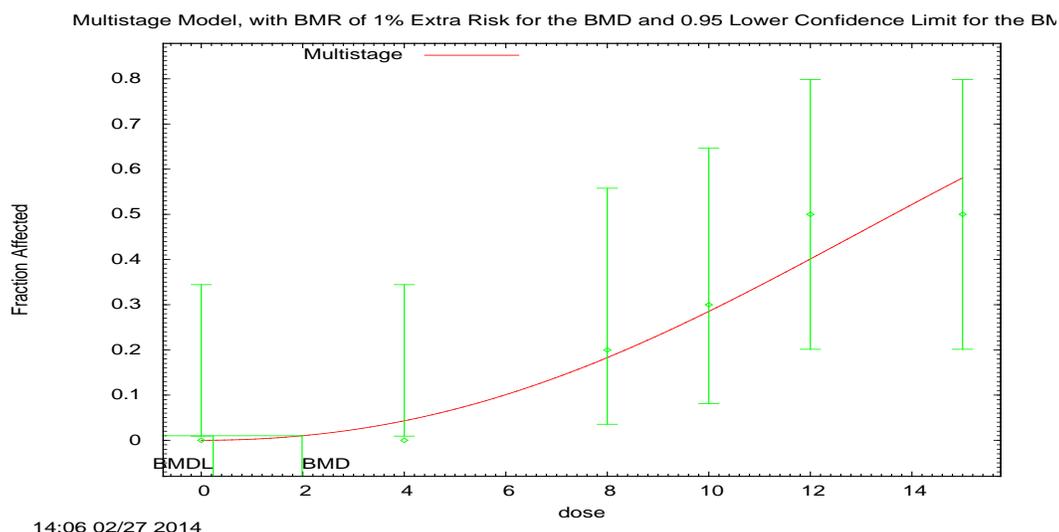
| Dose | Est. prob. | Expected | Observed | Size | Scaled residuals |
|------|------------|----------|----------|------|------------------|
| 0 | 0 | 0 | 0 | 10 | 0 |
| 4 | 0.1418 | 1.418 | 0 | 10 | -1.286 |
| 8 | 0.2635 | 2.635 | 2 | 10 | -0.456 |
| 10 | 0.3178 | 3.178 | 3 | 10 | -0.121 |
| 12 | 0.368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

Chi² = 2.79 d.f. = 5 p-value = 0.7325

1 **Table D-5. Model predictions for convulsions in female F344 rats exposed to**
 2 **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, so this model was excluded from consideration. Of the remaining 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 | |

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 5 -0.67, 0.14, 0.11, 0.64, and -0.51, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 5.98 and
 6 2.47 mg/kg-day, respectively.



7
 8 BMR= 1% ER; dose shown in mg/kg-day.

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

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

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

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

Benchmark Dose Computation

BMR = 1% ER

BMD = 1.98616

BMDL at the 95% confidence level = 0.235433

Parameter Estimates

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

Analysis of Deviance Table

| Model | Log (likelihood) | Number of parameters | Deviance | Test d.f. | p-value |
|---------------|------------------|----------------------|----------|-----------|----------|
| Full model | -24.9756 | 6 | | | |
| Fitted model | -25.7624 | 2 | 1.57351 | 4 | 0.8135 |
| Reduced model | -33.7401 | 1 | 17.529 | 5 | 0.003598 |

AIC: = 55.5247

Goodness-of-Fit Table

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

Chi² = 1.16 d.f. = 4 p-value = 0.8854

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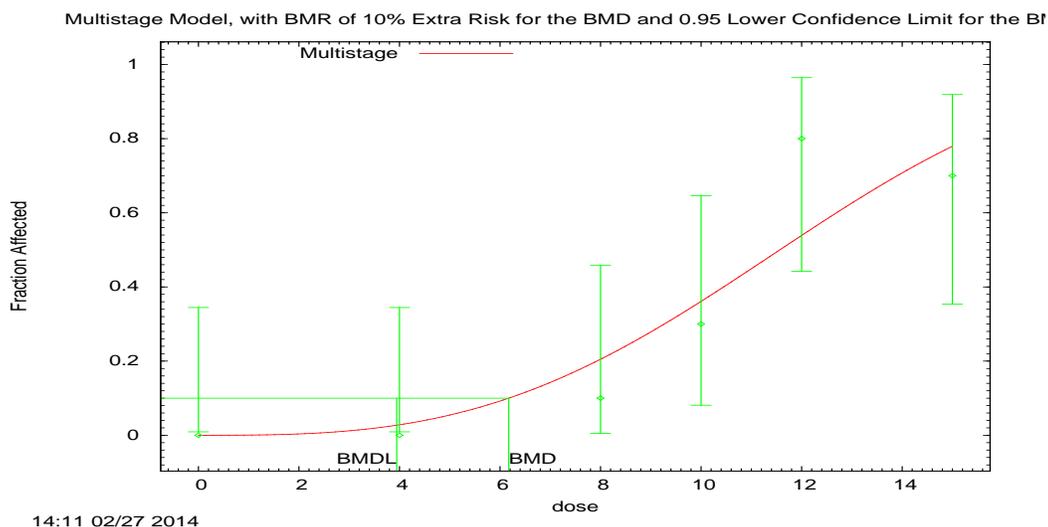
Table D-6. Model predictions for convulsions in male F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 10% ER

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

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^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00, -0.54, -0.82, -0.40, 1.66, and -0.61, respectively.

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



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BMR = 10% ER; dose shown in mg/kg-day.

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

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

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

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

Benchmark Dose Computation

BMR = 10% ER

BMD = 6.17392

BMDL at the 95% confidence level = 3.94501

Parameter Estimates

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

Analysis of Deviance Table

| Model | Log (likelihood) | Number of parameters | Deviance | Test d.f. | p-value |
|---------------|------------------|----------------------|----------|-----------|---------|
| Full model | -20.4721 | 6 | | | |
| Fitted model | -22.9013 | 1 | 4.85838 | 5 | 0.4334 |
| Reduced model | -37.4599 | 1 | 33.9755 | 5 | <0.0001 |

AIC: = 47.8027

Goodness-of-Fit Table

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

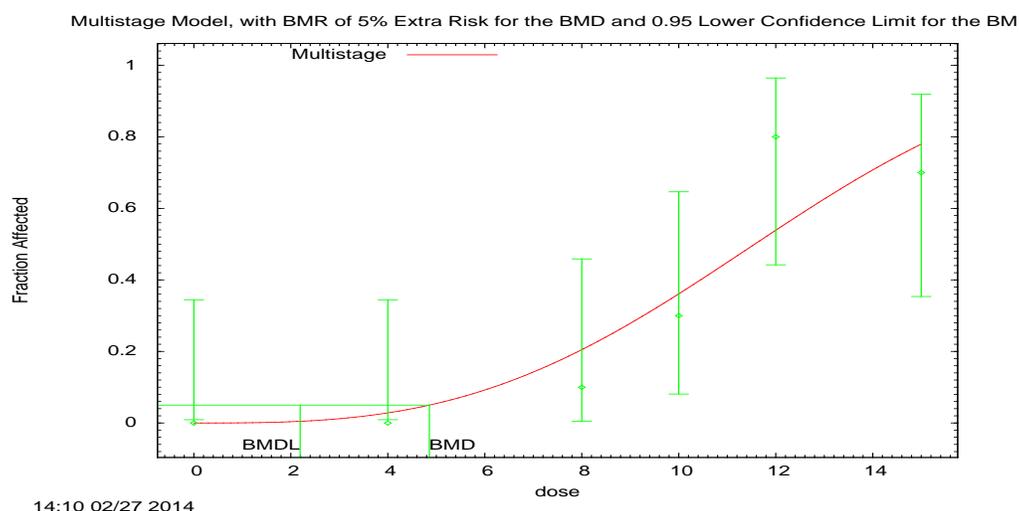
Chi² = 4.24 d.f. = 5 p-value = 0.5153

1 **Table D-7. Model predictions for convulsions in male F344 rats exposed to**
 2 **RDX by gavage for 90 days (Crouse et al., 2006); BMR = 5% ER**

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

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were
 5 0.000, -0.54, -0.82, -0.40, 1.66, and -0.61, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were
 6 6.17 and 3.95 mg/kg-day, respectively.

7 ^bFor the Multistage 5° model, the beta coefficient estimates were 0 (boundary of parameters space). The models
 8 in this row reduced to the Multistage 4° model.



9
 10 BMR = 5% ER; dose shown in mg/kg-day.

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

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

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

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

Benchmark Dose Computation

BMR = 5% ER

BMD = 4.85686

BMDL at the 95% confidence level = 2.19244

Parameter Estimates

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

Analysis of Deviance Table

| Model | Log (likelihood) | Number of parameters | Deviance | Test d.f. | p-value |
|---------------|------------------|----------------------|----------|-----------|---------|
| Full model | -20.4721 | 6 | | | |
| Fitted model | -22.9013 | 1 | 4.85838 | 5 | 0.4334 |
| Reduced model | -37.4599 | 1 | 33.9755 | 5 | <0.0001 |

AIC: = 47.8027

Goodness-of-Fit Table

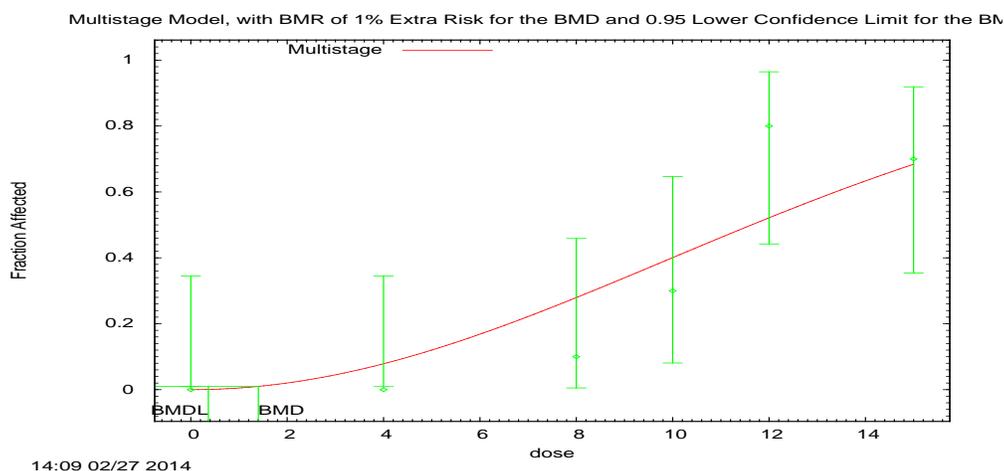
| Dose | Est. prob. | Expected | Observed | Size | Scaled residuals |
|------|------------|----------|----------|------|------------------|
| 0 | 0 | 0 | 0 | 10 | 0 |
| 4 | 0.0282 | 0.282 | 0 | 10 | -0.539 |
| 8 | 0.2049 | 2.049 | 1 | 10 | -0.822 |
| 10 | 0.3609 | 3.609 | 3 | 10 | -0.401 |
| 12 | 0.5387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

Chi² = 4.24 d.f. = 5 p-value = 0.5153

1 **Table D-8. Model predictions for convulsions in male F344 rats exposed to**
 2 **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 | 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 | |

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 5 -0.92, -1.26, -0.65, 1.76, and 0.11, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 4.54 and
 6 2.95 mg/kg-day, respectively.
 7 ^bThe Multistage 5° model may appear equivalent to the Multistage 4° model; however, differences exist in digits
 8 not displayed in the table.



9
 10 BMR = 1% ER; dose shown in mg/kg-day.

11 **Figure D-6. Plot of incidence rate by dose, with fitted curve for selected model,**
 12 **for convulsions in male F344 rats exposed to RDX by gavage for 90 days**
 13 **(Crouse et al., 2006).**

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

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

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

Benchmark Dose Computation

BMR = 1% ER

BMD = 1.40125

BMDL at the 95% confidence level = 0.363499

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 50.3345

Goodness-of-Fit Table

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

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

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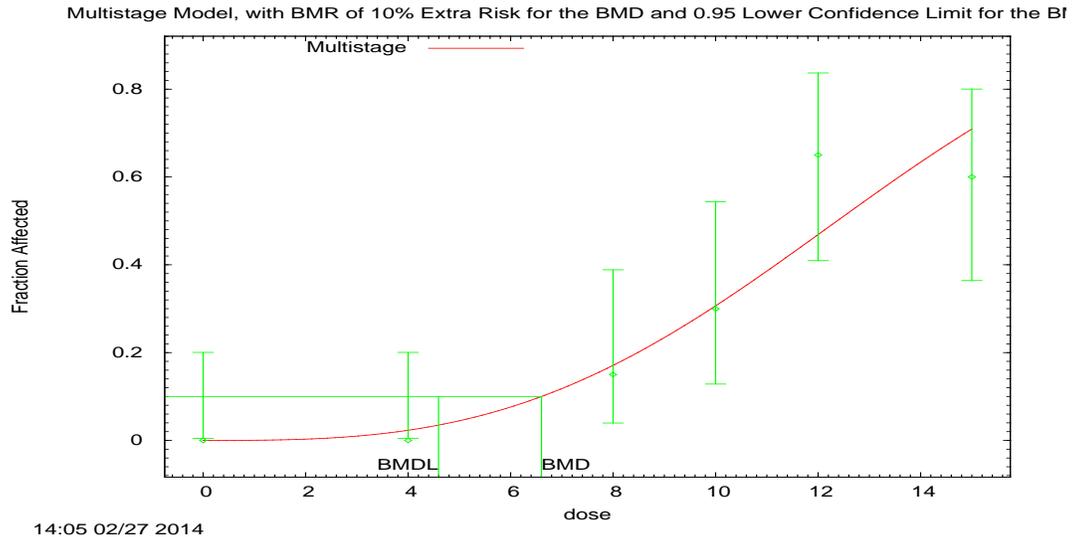
Table D-9. Model predictions for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006); BMR = 10% ER

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

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^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.

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



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BMR = 10% ER; dose shown in mg/kg-day.

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Figure D-7. Plot of incidence rate by dose, with fitted curve for selected model, for convulsions in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et al., 2006).

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

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 = 10% ER

BMD = 6.60247

BMDL at the 95% confidence level = 4.59346

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 100.913

Goodness-of-Fit Table

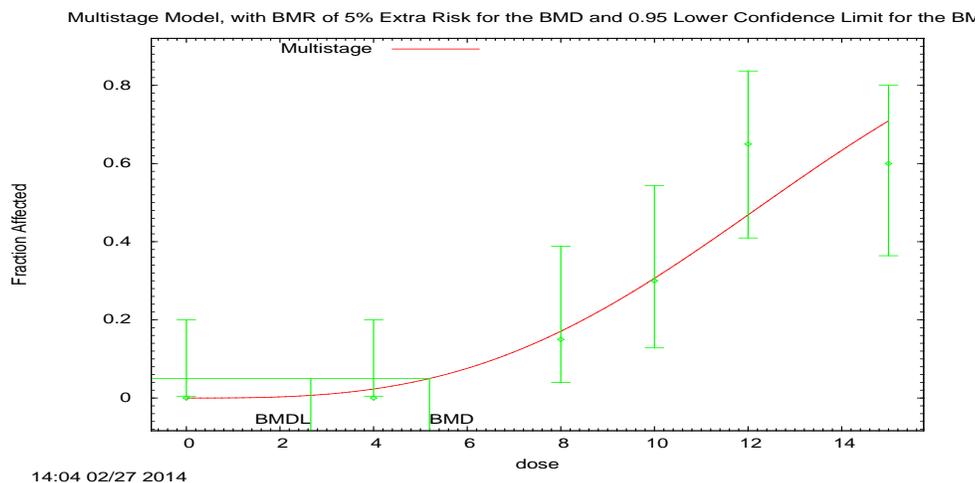
| Dose | Est. prob. | Expected | Observed | Size | Scaled residuals |
|------|------------|----------|----------|------|------------------|
| 0 | 0 | 0 | 0 | 20 | 0 |
| 4 | 0.0232 | 0.463 | 0 | 20 | -0.689 |
| 8 | 0.1709 | 3.418 | 3 | 20 | -0.248 |
| 10 | 0.3065 | 6.131 | 6 | 20 | -0.063 |
| 12 | 0.4688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

Chi² = 4.34 d.f. = 5 p-value = 0.5021

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

| Model ^a | Goodness of fit | | BMD _{5Pct} (mg/kg-d) | BMDL _{5Pct} (mg/kg-d) | Basis for model selection |
|----------------------------|-----------------|---------------|----------------------------------|-----------------------------------|--|
| | p-value | AIC | | | |
| Gamma | 0.484 | 101.79 | 5.78 | 3.80 | Of the models that provided an adequate fit, the Multistage 3° model was selected based on lowest AIC. |
| Logistic | 0.231 | 104.55 | 5.13 | 3.49 | |
| LogLogistic | 0.512 | 101.66 | 5.74 | 3.85 | |
| Probit | 0.291 | 103.61 | 5.29 | 3.43 | |
| LogProbit | 0.557 | 101.25 | 6.01 | 4.20 | |
| Weibull | 0.369 | 102.91 | 5.11 | 3.18 | |
| Multistage 2° | 0.364 | 103.03 | 3.47 | 2.20 | |
| Quantal-Linear | 0.0369 | 111.56 | 1.13 | 0.860 | |
| Multistage 3° | 0.502 | 100.91 | 5.19 | 2.66 | |
| Multistage 4° | 0.502 | 100.91 | 5.19 | 2.65 | |
| Multistage 5 ^{ob} | | | | | |
| Dichotomous-Hill | 0.696 | 101.64 | 6.98 | 4.80 | |

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 5 -0.69, -0.25, -0.06, 1.62, and -1.08, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 6.60 and
 6 4.59 mg/kg-day, respectively.
 7 ^bFor the Multistage 5° model, the b5 coefficient estimate was 0 (boundary of parameters space). The models in
 8 this row reduced to the Multistage 4° model.



9
 10 BMR = 5% ER; dose shown in mg/kg-day.

11 **Figure D-8. Plot of incidence rate by dose, with the fitted curve of the selected**
 12 **model, for convulsions in male and female F344 rats exposed to RDX by**
 13 **gavage for 90 days (Crouse et al., 2006).**

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

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

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

Benchmark Dose Computation

BMR = 5% ER

BMD = 5.19399

BMDL at the 95% confidence level = 2.65815

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 100.913

Goodness-of-Fit Table

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

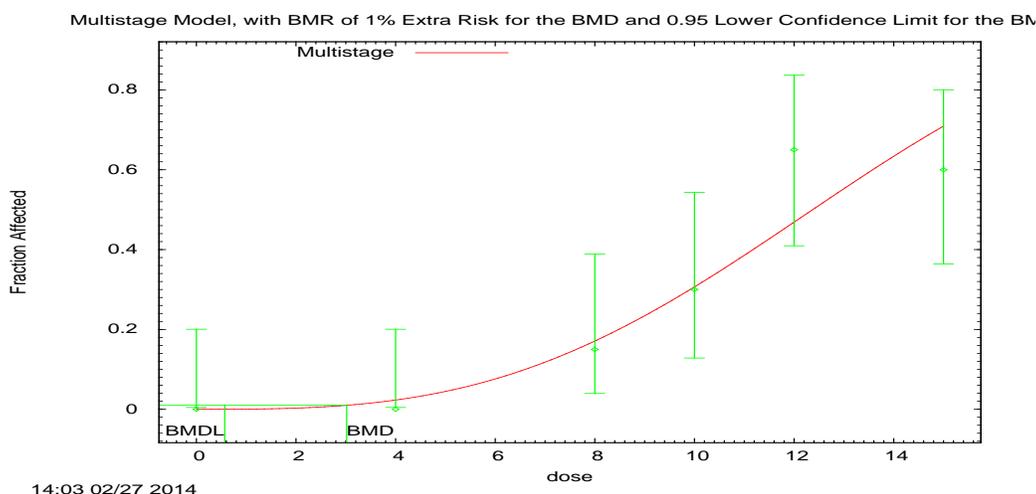
Chi^2 = 4.34 d.f. = 5 p-value = 0.5021

1 **Table D-11. Model predictions for convulsions in male and female F344 rats**
 2 **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 (p-value < 0.10), so it was excluded from consideration. 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). |
| 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 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0.00,
 5 -0.69, -0.25, -0.06, 1.62, and -1.08, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 6.60 and
 6 4.59 mg/kg-day, respectively.

7 ^bFor the Multistage 5° model, the beta coefficient estimates were 0 (boundary of parameters space). The models
 8 in this row reduced to the Multistage 4° model.
 9



11 **Figure D-9. Plot of incidence rate by dose, with the fitted curve of the**
 12 **multistage 3° model, for convulsions in male and female F344 rats exposed to**
 13 **RDX by gavage for 90 days (Crouse et al. 2006). BMR = 1% ER; dose shown in**
 14 **mg/kg-day.**

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

1 **Multistage Model.** (Version: 3.3; Date: 02/28/2013)
2 The form of the probability function is: $P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-$
3 $\text{beta1} * \text{dose}^{\text{1-beta2} * \text{dose}^2} \dots)]$
4
5

6 **Benchmark Dose Computation.**
7 BMR = 1% Extra risk
8 BMD = 3.01676
9 BMDL at the 95% confidence level = 0.569284
10
11

12 **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 |

13
14 **Analysis of Deviance Table**

| Model | Log(likelihood) | # Param's | 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 | <.0001 |

15
16 AIC: = 100.913
17

18 **Goodness of Fit Table**

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

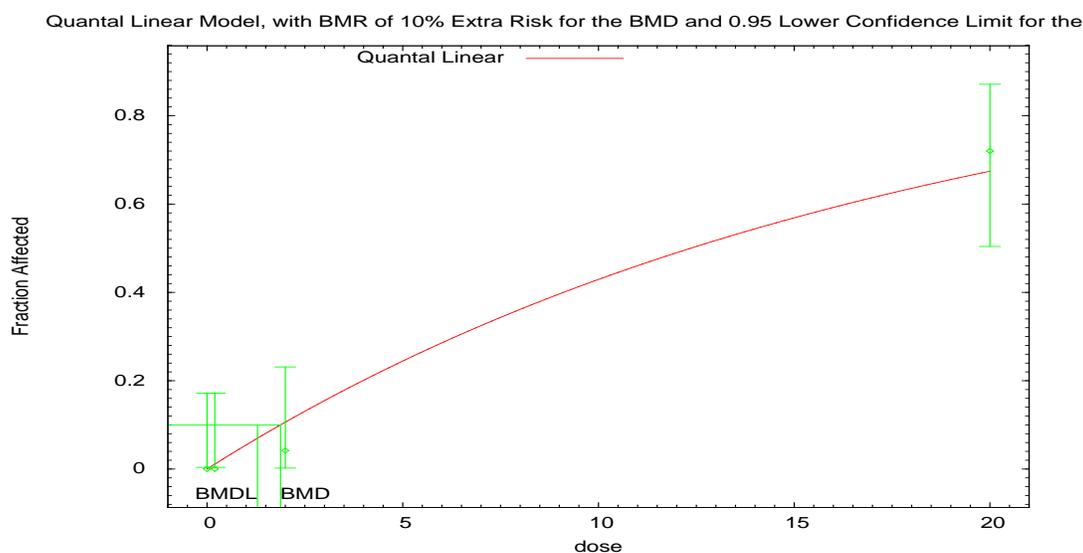
19
20 $\text{Chi}^2 = 4.34$ d.f = 5 P-value = 0.5021
21
22
23

1 **Table D-12. Model predictions for convulsions in female F344 rats exposed to**
 2 **RDx by gavage on gestational days (GDs) 6–19 (Cholakis et al. 1980);**
 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 | 0.989 | 42.003 | 3.62 | 1.56 | The Quantal-Linear model is selected based on lowest BMDL (BMDLs differed by more than threefold). |
| Logistic | 0.526 | 43.556 | 8.92 | 6.14 | |
| LogLogistic | 0.991 | 41.996 | 3.45 | 1.53 | |
| Probit | 0.577 | 43.348 | 7.64 | 5.39 | |
| LogProbit | 1.000 | 41.963 | 3.13 | 1.51 | |
| Weibull | 0.983 | 42.026 | 3.81 | 1.55 | |
| Multistage 3 ^{°b} | 0.960 | 42.113 | 4.26 | 1.54 | |
| Multistage 2 [°] | 0.960 | 42.113 | 4.26 | 1.54 | |
| Quantal-Linear | 0.669 | 42.077 | 1.88 | 1.29 | |

4
 5 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-day were 0.00,
 6 –0.52, –1.03, and 0.49, respectively.

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



9
 10 BMR = 10% ER; dose shown in mg/kg-day.

11 **Figure D-10. Plot of incidence rate by dose, with the fitted curve of the**
 12 **selected model, for convulsions in female F344 rats exposed to RDX by gavage**
 13 **on GDs 6–19 (Cholakis et al. 1980).**
 14

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

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

Benchmark Dose Computation

BMR = 10% ER

BMD = 1.87886

BMDL at the 95% confidence level = 1.28909

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 42.0769

Goodness-of-Fit Table

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

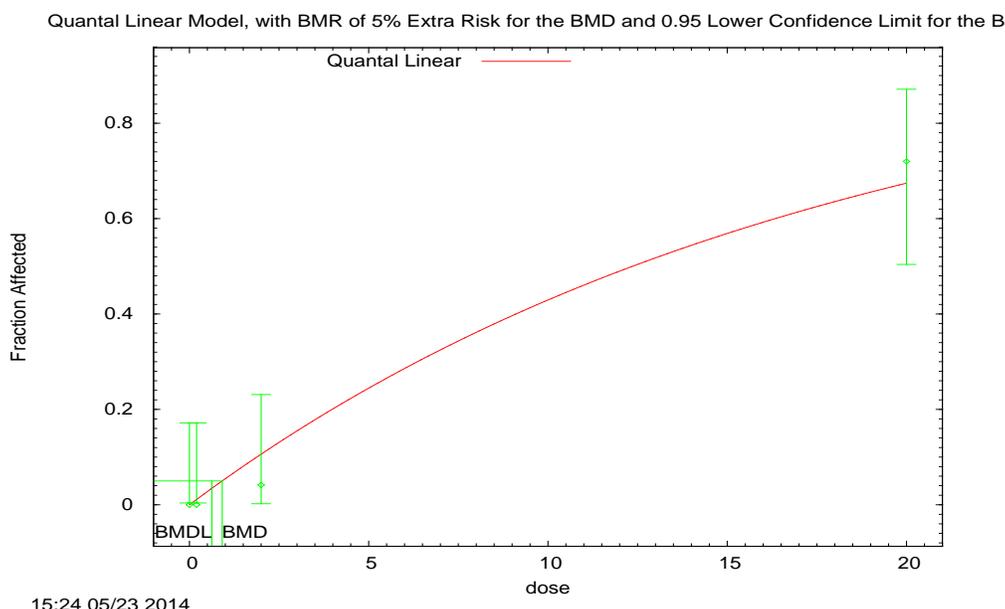
Chi^2 = 1.56 d.f. = 3 p-value = 0.6686

1 **Table D-13. Model predictions for convulsions in female F344 rats exposed to**
 2 **RDX by gavage on GDs 6–19 (Cholakis et al., 1980); BMR = 5% ER**

| Model ^a | Goodness of fit | | BMD _{5Pct} (mg/kg-d) | BMDL _{5Pct} (mg/kg-d) | Basis for model selection |
|----------------------------|-----------------|---------------|----------------------------------|-----------------------------------|--|
| | p-value | AIC | | | |
| Gamma | 0.989 | 42.003 | 2.31 | 0.759 | The Quantal-Linear model is selected based on lowest BMDL (BMDLs differed by more than threefold). |
| Logistic | 0.526 | 43.556 | 6.53 | 3.90 | |
| LogLogistic | 0.991 | 41.996 | 2.27 | 0.823 | |
| Probit | 0.577 | 43.348 | 5.41 | 3.34 | |
| LogProbit | 1.000 | 41.963 | 2.18 | 0.902 | |
| Weibull | 0.983 | 42.026 | 2.36 | 0.756 | |
| Multistage 2° | 0.960 | 42.113 | 2.51 | 0.747 | |
| Quantal-Linear | 0.669 | 42.077 | 0.915 | 0.628 | |
| Multistage 3° ^b | 0.960 | 42.113 | 2.51 | 0.747 | |

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-day were 0.00,
 5 –0.52, –1.03, and 0.49, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 1.88 and
 6 1.29 mg/kg-day, respectively.

7 ^bThe Multistage 3° model may appear equivalent to the Multistage 2° model; however, differences exist in digits
 8 not displayed in the table.



9
 10 BMR = 5% ER; dose shown in mg/kg-day.

11 **Figure D-11. Plot of incidence rate by dose, with the fitted curve of the**
 12 **selected model, for convulsions in female F344 rats exposed to RDX by gavage**
 13 **on GDs 6–19 (Cholakis et al., 1980).**

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

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

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

Benchmark Dose Computation

BMR = 5% ER

BMD = 0.914694

BMDL at the 95% confidence level = 0.627577

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 42.0769

Goodness-of-Fit Table

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

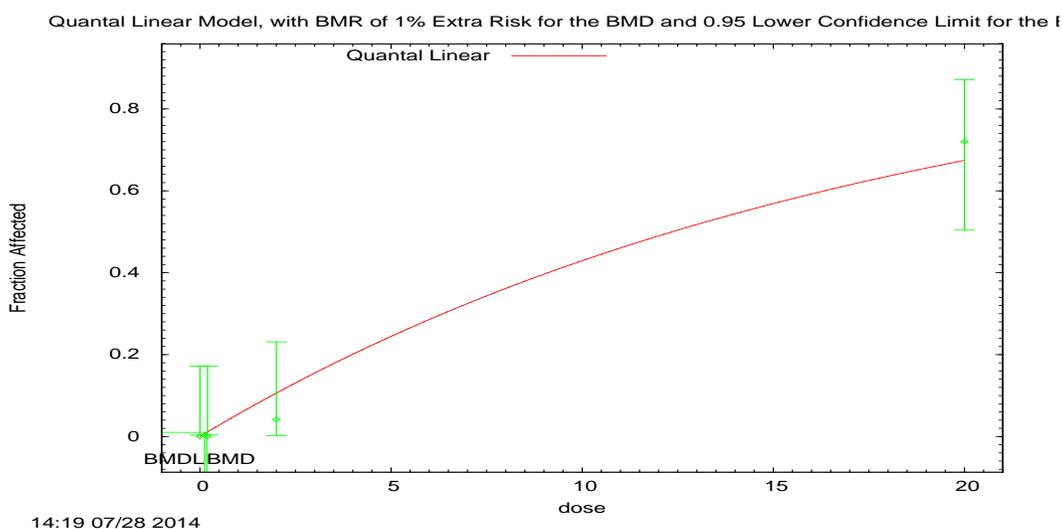
Chi² = 1.56 d.f. = 3 p-value = 0.6686

1 **Table D-14. Model predictions for convulsions in female F344 rats exposed to**
 2 **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 | 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 | |

3
 4 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.2, 2, and 20 mg/kg-day were 0.00,
 5 –0.52, –1.03, and 0.49, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 1.88 and
 6 1.29 mg/kg-day, respectively.

7 ^bThe Multistage 3[°] model may appear equivalent to the Multistage 2[°] model; however, differences exist in digits
 8 not displayed in the table.
 9



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

12 **Figure D-12. Plot of incidence rate by dose, with the fitted curve of the**
 13 **selected model, for convulsions in female F344 rats exposed to RDX by gavage**
 14 **on GDs 6–19 ([Cholakis et al., 1980](#)).**

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

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

Benchmark Dose Computation

BMR = 1% ER

BMD = 0.179224

BMDL at the 95% confidence level = 0.122966

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 42.0769

Goodness-of-Fit Table

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

Chi² = 1.56 d.f. = 3 p-value = 0.6686

1 **Table D-15. Model predictions for combined incidence of convulsion and**
 2 **mortality in male and female F344 rats exposed to RDX by gavage for 90 days**
 3 **(Crouse et al., 2006); BMR = 1% ER**

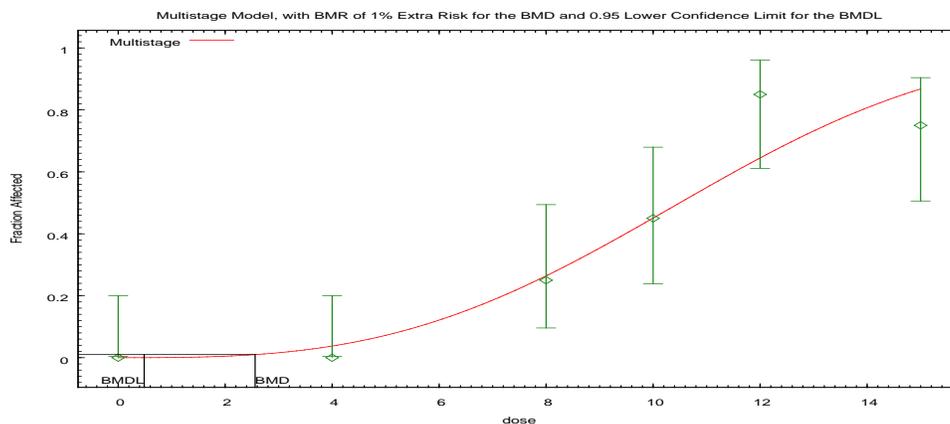
| Model ^a | Goodness of fit | | BMD _{1Pct} (mg/kg-d) | BMDL _{1Pct} (mg/kg-d) | Basis for model selection |
|----------------------------|-----------------|--------|----------------------------------|-----------------------------------|---|
| | p-value | AIC | | | |
| Gamma | 0.245 | 99.260 | 3.73 | 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 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 | |

4
 5 ^aSelected model in bold; scaled residuals for selected model for doses 0, 4, 8, 10, 12, and 15 mg/kg-day were 0,
 6 -0.88, -0.14, -0.01, 1.92, -1.55, respectively.

7 ^bThe Multistage 4° model may appear equivalent to the Multistage 3° model; however, differences exist in digits
 8 not displayed in the table.

9 ^cThe Multistage 3° model may appear equivalent to the Multistage 4° model; however, differences exist in digits
 10 not displayed in the table.

11



12
 13 BMR = 1% ER; dose shown in mg/kg-day.

14 **Figure D-13. Plot of incidence rate by dose with fitted curve for Multistage 3°**
 15 **model for Model predictions for combined incidence of convulsion and**
 16 **mortality in male and female F344 rats exposed to RDX by gavage for 90 days**
 17 **(Crouse et al., 2006).**

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: $P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose} - \text{beta}2 * \text{dose}^2 \dots)]$

Benchmark Dose Computation.

BMR = 1% Extra risk

BMD = 2.56012

BMDL at the 95% confidence level = 0.486284

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 99.1817

Goodness-of-Fit Table

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

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

1 **Male Reproductive Effects**

2 Table D-16 (and Figure D-14) presents the BMD modeling results for incidence of testicular
 3 degeneration for male B6C3F₁ mice based on data from [Lish et al. \(1984\)](#), using a BMR of 10% ER.

4 **Table D-16. Model predictions for testicular degeneration in male B6C3F₁**
 5 **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). |
| Logistic | 0.159 | 103.40 | 97.1 | 66.1 | |
| LogLogistic | 0.377 | 100.91 | 63.6 | 32.3 | |
| Probit | 0.178 | 103.12 | 93.1 | 61.4 | |
| LogProbit | 0.876 | 97.564 | 56.0 | 16.3 | |
| Multistage 2 ^{°c} Multistage 3 [°] Multistage 4 [°] | 0.357 | 101.10 | 66.5 | 35.4 | |

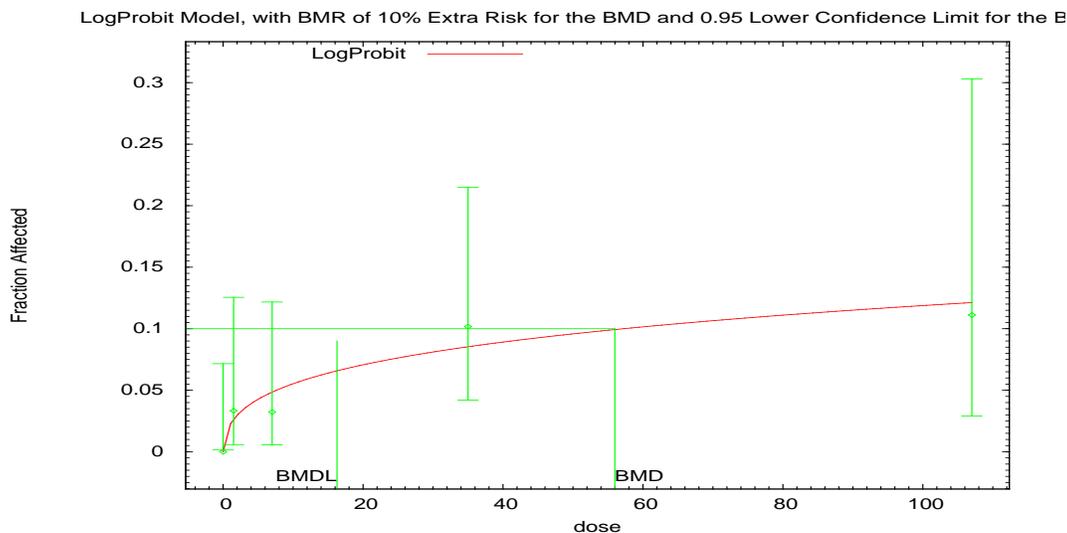
6
 7 ^aSelected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-ay were 0.00,
 8 0.32, -0.61, 0.43, and -0.17, respectively.

9 ^bFor the Gamma and Weibull models, the power parameter estimates were 1 (boundary of parameter space). The
 10 models in this row are equivalent to the Quantal-Linear model.

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

14

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



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BMR = 10% ER; dose shown in mg/kg-day.

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

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

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

Slope parameter is not restricted

Benchmark Dose Computation

BMR = 10% ER

BMD = 55.9784

BMDL at the 95% confidence level = 16.2787

Parameter Estimates

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

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 | -46.4212 | 5 | | | |
| Fitted model | -46.7817 | 2 | 0.721088 | 3 | 0.8682 |
| Reduced model | -52.1663 | 1 | 11.4902 | 4 | 0.02157 |

2
3 AIC: = 97.5635

4
5 **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 |

6
7 $\chi^2 = 0.69$ d.f. = 3 p -value = 0.8759

1 **Kidney Effects**

2 Table D-17 (and Figure D-15) presents the BMD model results for incidence of suppurative
 3 inflammation of the prostate in male F344 rats based on data from [Levine et al. \(1983b\)](#), using a
 4 BMR of 10% ER.

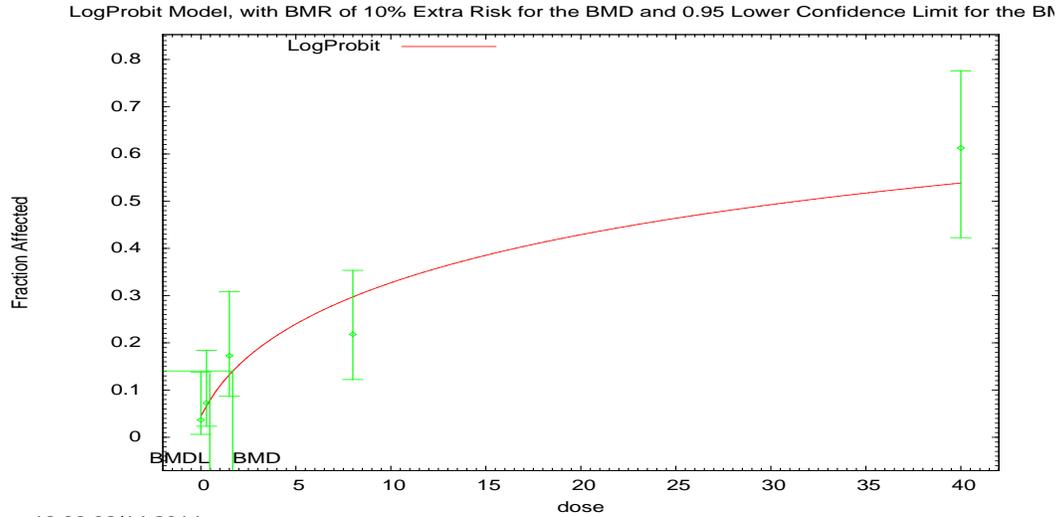
5 **Table D-17. Model predictions for prostate suppurative inflammation in male**
 6 **F344 rats exposed to RDX by diet for 24 months ([Levine et al., 1983b](#));**
 7 **BMR = 10% ER**

| 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). |
| 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 | |

8
 9 ^aSelected model in bold; scaled residuals for selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were
 10 -0.289, 0.172, 0.846, -1.298, and 0.819, respectively.

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

14 ^cThe Weibull model may appear equivalent to the Quantal-Linear model; however, differences exist in digits not
 15 displayed in the table.
 16



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BMR = 10% ER; dose shown in mg/kg-day.

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Figure D-15. 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., 1983b).

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

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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 distribution function

9

10

Slope parameter is not restricted

11

12

13

Benchmark Dose Computation

14

BMR = 10% ER

15

BMD = 1.67454

16

BMDL at the 95% confidence level = 0.468688

17

18

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 |

19

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-18).

12 **Table D-18. Mortality data selected for dose-response modeling for RDX**

| Reference | Species/sex | Dose | Incidence / Total (%) or Mean ± SD (no. 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 (no. 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. (1983b) (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-week study) | Male F344 rat | 0 mg/kg-d 10 14 20 28 40 | 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%) |
| (Crouse et al. (2006)) | Female F344 rat | 0 mg/kg-d 4 8 | 0 / 10 (0%) 0 / 10 (0%) 1 / 10 (20%) |

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

| Reference | Species/sex | Dose | Incidence / Total (%) or Mean ± SD (no. of animals) | |
|--|---|-----------|---|--------------|
| | | 10 | 2 / 10 (20%) | |
| | | 12 | 5 / 10 (50%) | |
| | | 15 | 4 / 10 (40%) | |
| | Male F344 rat | 0 mg/kg-d | 4 | 0 / 10 (0%) |
| | | | 8 | 0 / 10 (0%) |
| | | | 10 | 1 / 10 (10%) |
| | | | 12 | 3 / 10 (30%) |
| | | | 15 | 2 / 10 (20%) |
| | | | 15 | 3 / 10 (30%) |
| | Male and female F344 rat, combined | 0 mg/kg-d | 4 | 0 / 20 (0%) |
| | | | 8 | 0 / 20 (0%) |
| | | | 10 | 2 / 20 (10%) |
| | | | 12 | 5 / 20 (25%) |
| | | | 15 | 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%) | |

^aFor [Levine et al. \(1981a\)](#) and [Crouse et al. \(2006\)](#), the incidence rates across doses were determined to be not statistically significantly different between the males and females using an exact Cochran-Mantel-Haenszel test ($p \geq 0.10$). The data were combined across sex for each of these endpoints prior to modeling.

^bFor [von Oettingen et al. \(1949\)](#), one mortality was reported in the 15 mg/kg-d dose group. However, this mortality was most likely not related to RDX, so the animal that died was excluded.

^cFor [Cholakis et al. \(1980\)](#), one accidental death was reported in the 0 mg/kg-d dose group. The animal that died was excluded.

1
2 Tables D-19 to D-22 present the BMD modeling results for incidence of mortality from
3 [Crouse et al. \(2006\)](#), [von Oettingen et al. \(1949\)](#), [Levine et al. \(1983b\)](#), and [Angerhofer et al. \(1986\)](#).
4 The following datasets were not modeled because each had either no response or a positive
5 response only in the highest dose group: 11-week mortality from [Lish et al. \(1984\)](#), both male (one
6 death in control group) and female; 13-week mortality data from [Levine et al. \(1983b\)](#), both male
7 and female; mortality in female CD rats and male and female F344 rats from [Cholakis et al. \(1980\)](#);
8 and mortality in female F344 rats during gestational exposure from [Cholakis et al. \(1980\)](#).

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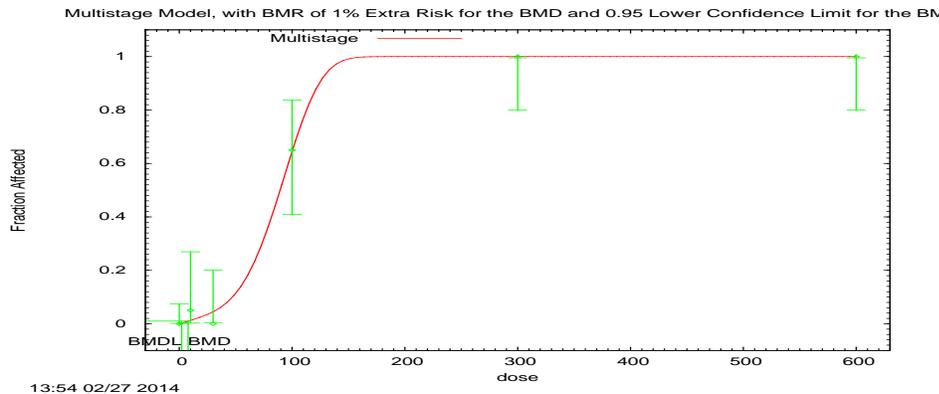
1 **Table D-19. Model predictions for combined mortality in male and female**
 2 **F344 rats exposed to RDX by diet for 13 weeks (Levine et al., 1981); BMR = 1%**
 3 **ER**

| Model ^a | Goodness of fit | | BMD _{1Pct} (mg/kg-day) | BMDL _{1Pct} (mg/kg-day) | 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 | |

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

^bThe parameter convergence parameter was increased to 2e-08 to obtain convergence.

4



5

6 **Figure D-16. Plot of incidence rate by dose, with the fitted curve of the**
 7 **multistage 2° model, for combined mortality in male and female F344 rats**
 8 **exposed to RDX by diet for 13 weeks (Levine et al., 1981); BMR = 1% ER.**

9

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

1 **Multistage Model.** (Version: 3.3; Date: 02/28/2013)
 2 The form of the probability function is: $P[\text{response}] = \text{background} + (1-\text{background}) * [1 - \text{EXP}(-$
 3 $\text{beta1} * \text{dose}^{\text{1-beta2} * \text{dose}^2} \dots)]$
 4
 5

6 **Benchmark Dose Computation.**

7 BMR = 1% Extra risk
 8 BMD = 7.85287
 9 BMDL at the 95% confidence level = 2.15059
 10
 11

12 **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 |

13
 14 **Analysis of Deviance Table**

| Model | Log(likelihood) | # Param's | 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 | <.0001 |

15
 16 AIC: = 40.9353
 17

18 **Goodness of Fit Table**

| Dose | Est. Prob. | Expected | Observed | Size | Scaled Resid |
|------|------------|----------|----------|------|--------------|
| 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 |

19
 20 $\text{Chi}^2 = 3.14$ d.f = 4 P-value = 0.5352
 21

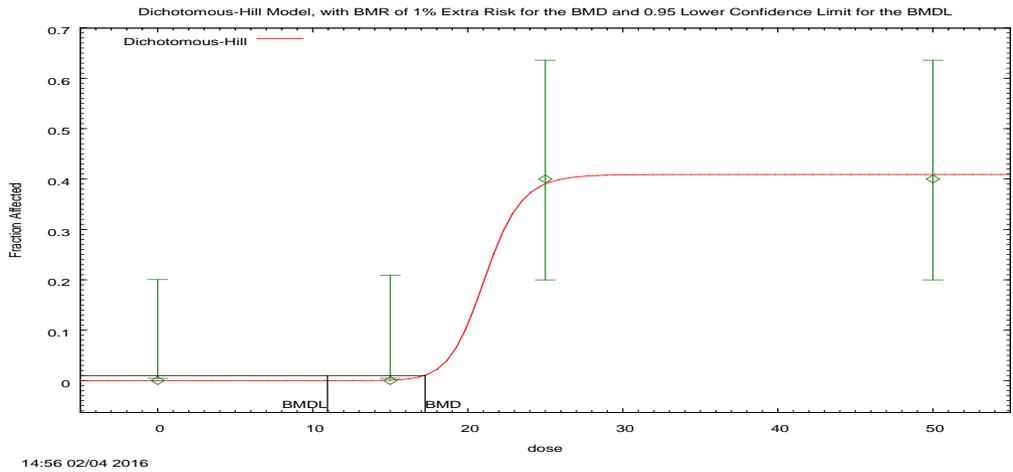
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Table D-20. Summary of BMD modeling results for model predictions for mortality (number found dead) in rats exposed to RDX in the diet for 13 weeks (von Oettingen et al., 1949)

| Model ^a | Goodness of fit | | BMD _{1Pct} (mg/kg-day) | BMDL _{1Pct} (mg/kg-day) | Basis for model selection |
|----------------------------|-----------------|--------|------------------------------------|-------------------------------------|---|
| | p-value | AIC | | | |
| Gamma | 0.0341 | 66.088 | 3.14 | 0.648 | All the models besides dichotomous-Hill had goodness-of-fit p-values less than 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 | |

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

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Figure D-17. 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.

11

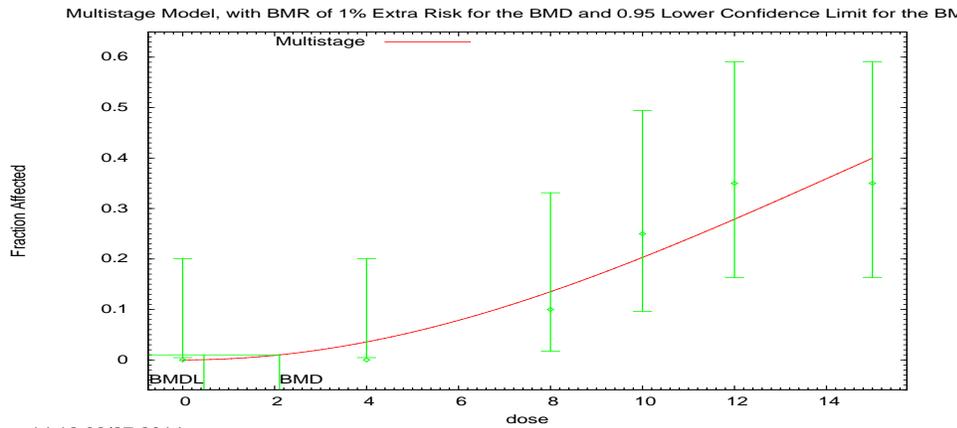
1 **Table D-21. Model predictions for combined mortality (number found dead)**
 2 **in male and female F344 rats exposed to RDX by gavage for 90 days (Crouse et**
 3 **al., 2006); BMR= 1% ER**

| Model ^a | Goodness of fit | | BMD _{1Pct} (mg/kg-day) | BMDL _{1Pct} (mg/kg-day) | 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 | |

^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 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



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3 **Figure D-18. 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 **RD_X 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: $P[\text{response}] = \text{background} + (1-\text{background}) * [1-\text{EXP}(-$
 9 $\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

10

11

12 **Benchmark Dose Computation.**

13 BMR = 1% Extra risk

14 BMD = 2.10625

15 BMDL at the 95% confidence level = 0.462994

16

17

18 **Parameter Estimates**

| Variable | Estimate | Default Initial Parameter Values |
|------------|------------|----------------------------------|
| Background | 0 | 0 |
| Beta(1) | 0 | 0.0134587 |
| Beta(2) | 0.00226548 | 0.00141278 |

19

20 **Analysis of Deviance Table**

| Model | Log(likelihood) | # Param's | 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 |

21

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

1 AIC: = 91.926

2

3 **Goodness of Fit Table**

| Dose | Est. Prob. | Expected | Observed | Size | Scaled Resid |
|------|------------|----------|----------|------|--------------|
| 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 |

4

5 Chi² = 1.94 d.f = 5 P-value = 0.8576

6

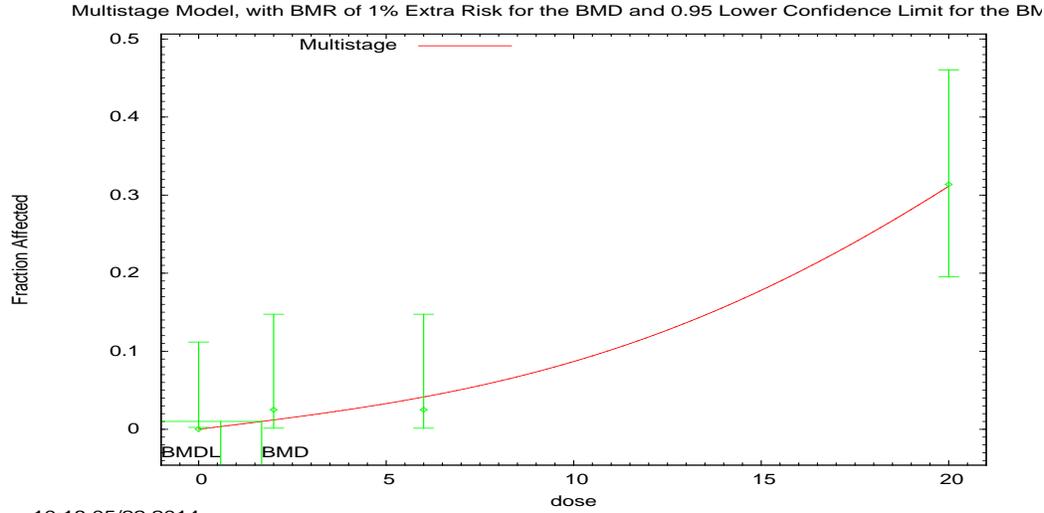
7 **Table D-22. Model predictions for mortality in female Sprague-Dawley rats**
 8 **exposed to RDX by gavage on gestation days 6–15 (Angerhofer, 1986); BMR =**
 9 **1% ER**

| Model ^a | Goodness of fit | | BMD _{1Pct} (mg/kg-day) | BMDL _{1Pct} (mg/kg-day) | Basis for model selection |
|----------------------|-----------------|---------------|------------------------------------|-------------------------------------|--|
| | p-value | AIC | | | |
| Gamma | 0.314 | 89.496 | 5.15 | 0.538 | 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 | 0.667 | 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 | |

^aSelected model in bold; scaled residuals for selected model for doses 0, 2, 6, and 20 mg/kg-d were 0.00, 0.76, -0.52, and 0.04, respectively. The BMD₁₀ and BMDL₁₀ for the selected model were 10.9 and 6.09 mg/kg-d, respectively.

10

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



1

Figure D-19. 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, 1986); BMR = 1% ER.

5

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

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

9

Benchmark Dose Computation.

BMR = 1% Extra risk

BMD = 1.68097

BMDL at the 95% confidence level = 0.587568

15

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 |

18

Analysis of Deviance Table

| Model | Log(likelihood) | # Param's | Deviance | Test d.f. | p-value |
|--------------|------------------|-----------|----------|-----------|---------|
| Full model | -41.0771 | 4 | | | |
| Fitted model | -41.4531 | 2 | 0.752152 | 2 | 0.6866 |

19

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

| | | | | | |
|---------------|----------|---|---------|---|--------|
| Reduced model | -57.4292 | 1 | 32.7043 | 3 | <.0001 |
|---------------|----------|---|---------|---|--------|

AIC: = 86.9063

Goodness of Fit Table

| Dose | Est. Prob. | Expected | Observed | Size | Scaled Resid |
|------|------------|----------|----------|------|--------------|
| 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 |

Chi² = 0.85 d.f = 2 P-value = 0.6549

D.2. BENCHMARK DOSE MODELING SUMMARY FOR CANCER ENDPOINTS

The cancer endpoints in the mouse that were selected for dose-response modeling are presented in Table D-23. For each endpoint, the doses and tumor incidence data used for the modeling are presented.

Table D-23. 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%) |

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

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

First, to determine whether a time-to-tumor analysis was warranted, the survival curves were compared across dose groups for female mice in [Lish et al. \(1984\)](#) in the study to determine whether time of death should be incorporated in the dose-response analysis of tumors. A log-rank test on the Kaplan-Meier survival curves per dose was used to do the comparison, excluding

1 animals that died prior to week 11 when the dose was reduced in the high-dose group to
 2 100 mg/kg-day. The test yielded a nonsignificant result ($p = 0.51$), so a time-to-tumor analysis was
 3 not necessary for this study.

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

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

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

19 The BMD modeling results for mouse tumor data sets are provided in Tables D-24 to D-28
 20 (and Figures D-20 to D-24).

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

22 **Table D-24. Model predictions for combined alveolar/bronchiolar adenoma**
 23 **and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months**
 24 **(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^{ob} 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. |

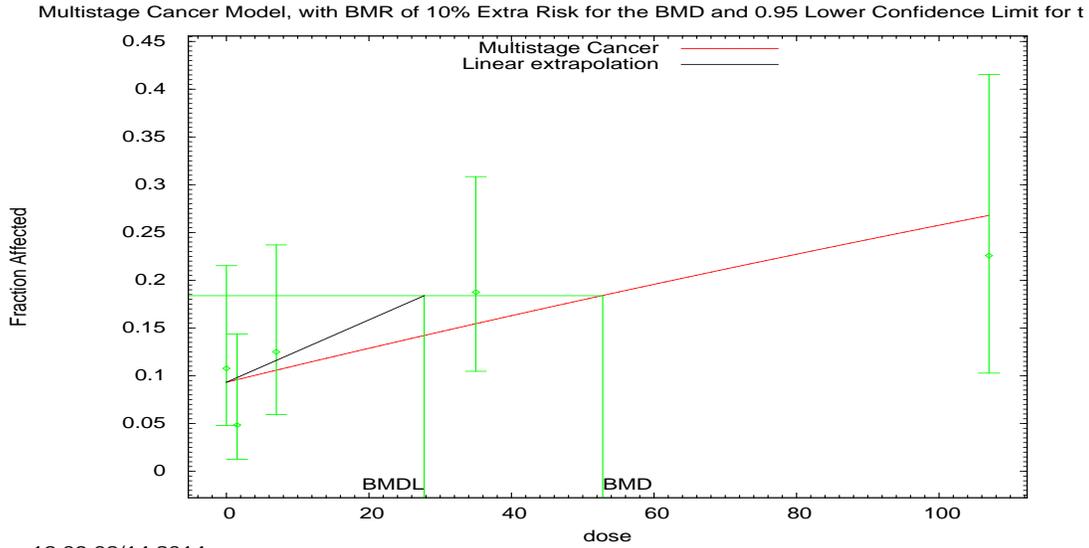
25
 26 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were
 27 0.40, -1.27, 0.50, 0.73, and -0.52, respectively.

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

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



2

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3

BMR = 10% ER; dose shown in mg/kg-day.

4

Figure D-20. 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).

7

8

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

9

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

10

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

11

The parameter betas are restricted to be positive

12

13

Benchmark Dose Computation

14

BMR = 10% ER

15

BMD = 52.8078

16

BMDL at the 95% confidence level = 27.748

17

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

18

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

19

Multistage Cancer Slope Factor = 0.00360387

20

21

Parameter Estimates

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

22

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 | -105.777 | 5 | | | |
| Fitted model | -107.341 | 2 | 3.12764 | 3 | 0.3724 |
| Reduced model | -110.164 | 1 | 8.77367 | 4 | 0.06701 |

2
3 AIC: = 218.682

4
5 **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 |

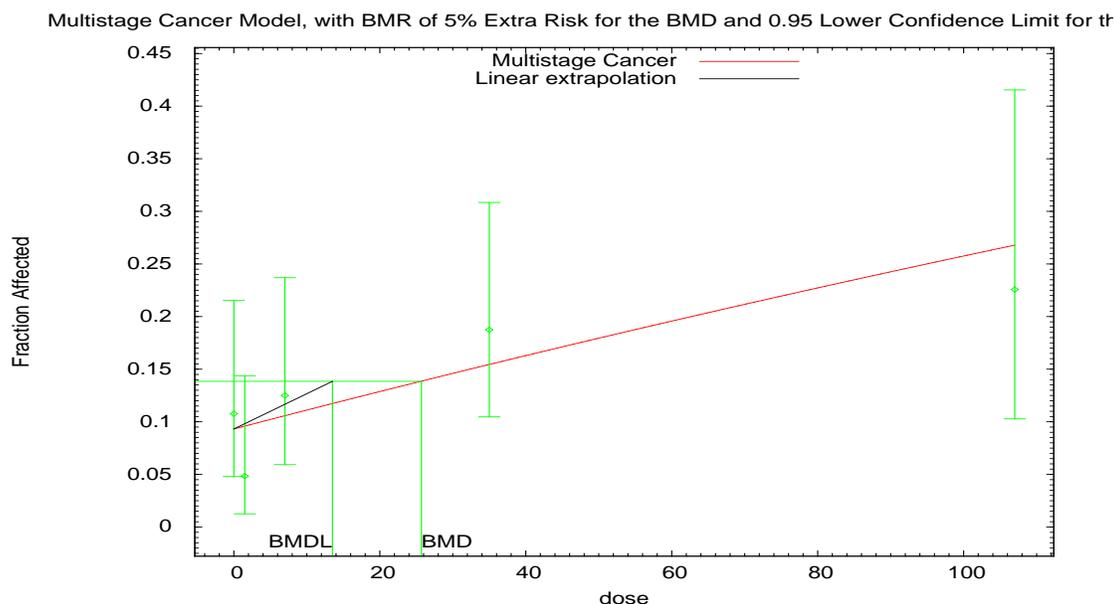
6
7 $\chi^2 = 2.84$ d.f. = 3 p -value = 0.4168

8

1 **Table D-25. Model predictions for combined alveolar/bronchiolar adenoma**
 2 **and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months**
 3 **(Lish et al., 1984); BMR = 5% ER**

| Model ^a | Goodness of fit | | BMD _{5Pct} (mg/kg-d) | BMDL _{5Pct} (mg/kg-d) | Basis for model selection |
|----------------------------------|-----------------|---------------|----------------------------------|-----------------------------------|---|
| | p-value | AIC | | | |
| Multistage 1^{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 for the selected model were
 7 52.8 and 27.7 mg/kg-day, respectively.
 8



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10 BMR = 5% ER; dose shown in mg/kg-day.

11 **Figure D-21. Plot of incidence rate by dose, with fitted curve for selected**
 12 **model, for combined alveolar/bronchiolar adenoma and carcinoma in female**
 13 **B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al., 1984).**

14

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

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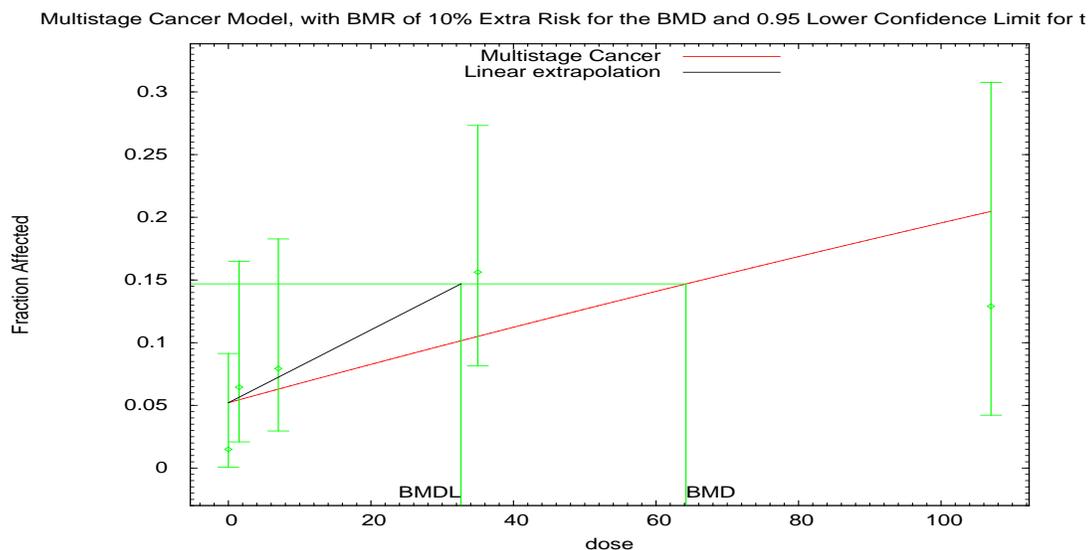
Table D-26. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months (Parker et al., 2006); BMR = 10% ER

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

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^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
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15

BMR = 10% ER; dose shown in mg/kg-day.

Figure D-22. 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: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2)]$

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 |

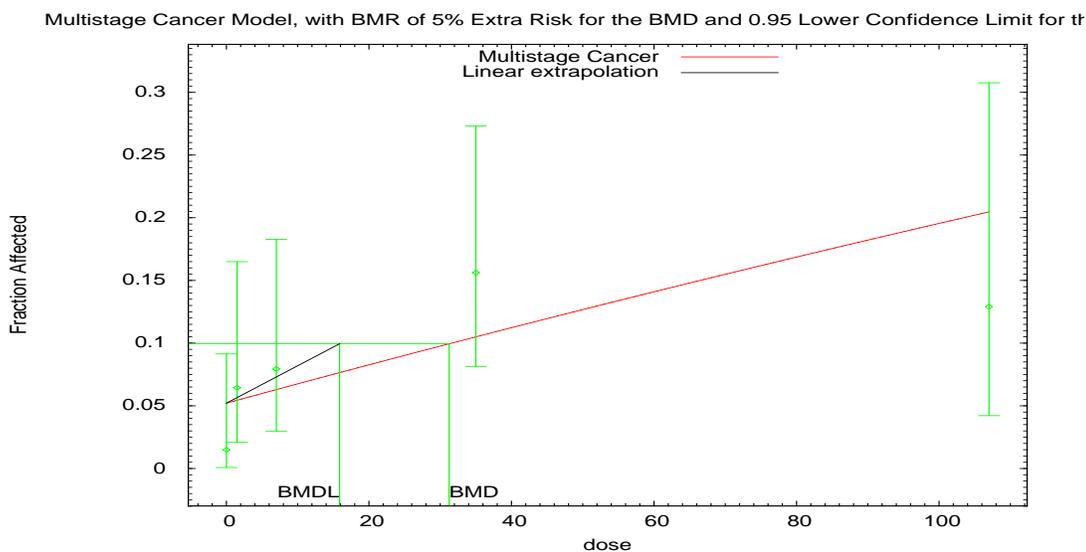
Chi² = 5.17 d.f. = 3 p-value = 0.16

1 **Table D-27. 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 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 BMR = 5% ER; dose shown in mg/kg-day.

13 **Figure D-23. Plot of incidence rate by dose, with fitted curve for selected**
 14 **model, for B6C3F₁ female mouse combined hepatocellular adenoma and**
 15 **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

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

2 The form of the probability function is: $P[\text{response}] = \text{background} +$
3 $(1 - \text{background}) * [1 - \text{EXP}(-\beta_1 * \text{dose} - \beta_2 * \text{dose}^2 \dots)]$

4 The parameter betas are restricted to be positive

6 **Benchmark Dose Computation**

7 BMR = 5% ER

8 BMD = 31.2563

9 BMDL at the 95% confidence level = 15.8846

10 BMDU at the 95% confidence level = 136.989

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

12 Multistage Cancer Slope Factor = 0.0031477

14 **Parameter Estimates**

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

16 **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 |

17 AIC: = 164.063

20 **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 |

21 $\chi^2 = 5.17$ 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 = 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

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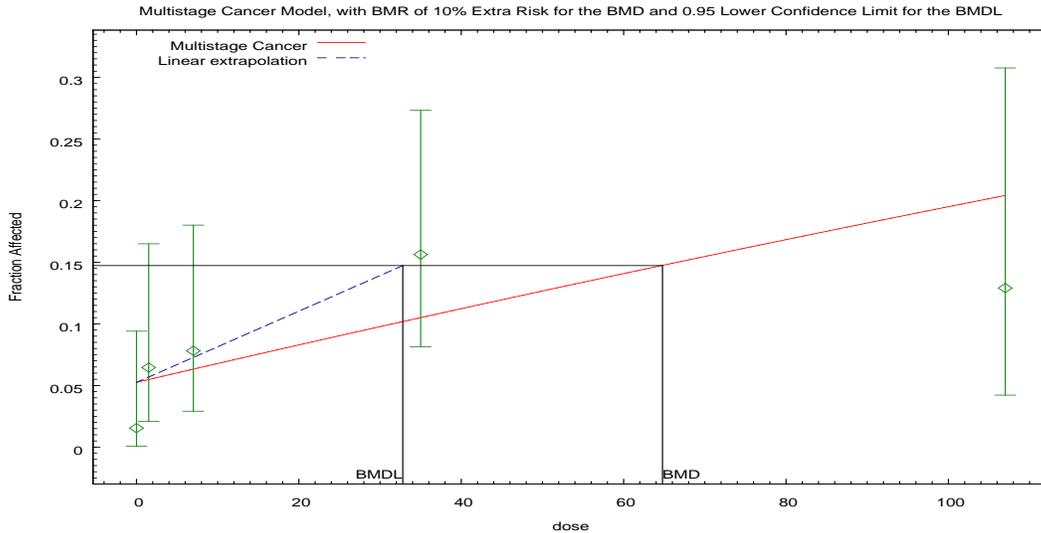
Table D-28. Model predictions for combined hepatocellular adenoma and carcinoma in female B6C3F₁ mice exposed to RDX by diet for 24 months, using incidence frequencies from [Parker et al. \(2006\)](#) and sample sizes from [Lish et al. \(1984\)](#); BMR = 10% ER

| 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. |

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



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BMR = 10% ER, dose shown in mg/kg-day.

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Figure D-24. Plot of incidence rate by dose with fitted curve for Multistage-Cancer 1^o 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 [\(Parker et al., 2006\)](#).

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: $P[\text{response}] = \text{background} + (1 - \text{background}) * [1 - \text{EXP}(-\text{beta}1 * \text{dose}^1 - \text{beta}2 * \text{dose}^2 \dots)]$

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 |

Chi^2 = 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. 1983b](#)) and incidence of lung carcinomas in male
 5 B3C6F₁ mice (Table D-29). The resulting candidate oral slope factors (OSFs) are presented for
 6 comparison with OSF estimates provided in Section 2.3.3 of the Toxicological Review.

7 **Table D-29. Liver carcinoma data from [Levine et al. \(1983b\)](#)**

| 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. (1983b) | 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-30; detailed BMD modeling results are provided in Table D-31
 20 (and Figure D-25).

1 **Table D-30. 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)**

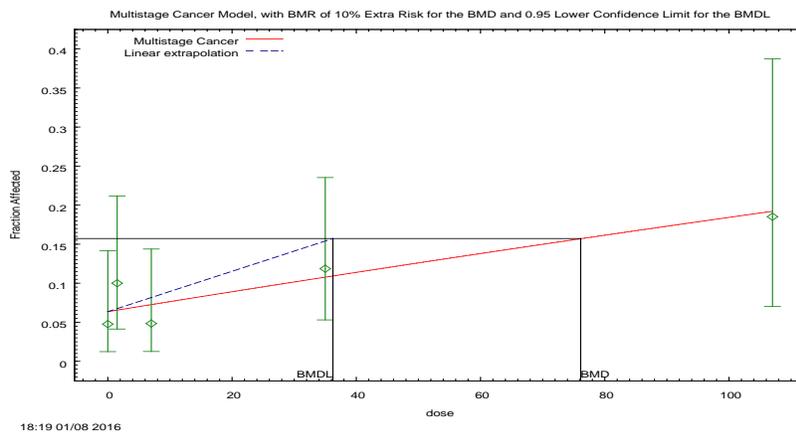
| Tumor type | Selected model | BMR | BMD, mg/kg-d | BMDL, mg/kg-d | POD = BMDL _{10-HED} ^a mg/kg-d | Candidate OSF ^b (mg/kg-d) ⁻¹ |
|---------------------------------|----------------|--------|--------------|---------------|---|--|
| Alveolar/bronchiolar carcinomas | Multistage 1° | 10% ER | 76.1 | 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-31. 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 | 0.561 | 162.00 | 76.1 | 36.2 | All of the models reduced to the multistage 1° model, so this model was selected. |
| Multistage 2° | | | | | |
| Multistage 3° | | | | | |
| Multistage 4° | | | | | |

11
 12 ^a Selected model in bold; scaled residuals for selected model for doses 0, 1.5, 7, 35, and 107 mg/kg-day were
 13 -0.52, 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



17
 18 BMR = 10% ER; dose shown in mg/kg-day.

19 **Figure D-25. Plot of incidence rate by dose with fitted curve for Multistage-**
 20 **Cancer 1° model for Model predictions for alveolar/bronchiolar carcinoma in**
 21 **male B6C3F₁ mice exposed to RDX by diet for 24 months (Lish et al. 1984).**

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

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

The parameter betas are restricted to be positive

Benchmark Dose Computation.

BMR = 10% Extra risk

BMD = 76.1197

BMDL at the 95% confidence level = 36.2316

BMDU at the 95% confidence level = 27443100

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

Multistage Cancer Slope Factor = 0.00276003

Parameter Estimates

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

Analysis of Deviance Table

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

AIC: = 162.001

Goodness-of-Fit Table

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

Chi² = 2.06 d.f. = 3 p-value = 0.561

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

11 Therefore, a non-time-dependent dose-response analysis was conducted using BMDS
12 multistage-cancer models. The model selection procedures described in Section D.2.1 were used to
13 select the appropriate models. To account for the difference in the survival curves across the
14 groups for rats, the number of animals alive at 12 months was used as the denominator in the
15 analysis (denominators listed in Table D-28). Because the maximum liver tumor response in the
16 male rat was 6.4%, a BMR of 5% was used to model male rat liver tumor data in order to obtain a
17 BMD and BMDL in the range of the experimental data, as recommended in Section 3.2 of *Guidelines*
18 *for Carcinogen Risk Assessment* ([U.S. EPA, 2005a](#)).

19 To estimate the HED at the BMDL, HEDs based on both administered dose scaled by $BW^{3/4}$
20 and physiologically based pharmacokinetic (PBPK) modeling were considered. Confidence in the
21 revised rat PBPK model is relatively high (see Appendix C, Section C.1.5); however, the choice of an
22 internal dose is not straightforward. First, evidence regarding the involvement of metabolites has
23 been discussed in the literature only in the context of the mouse, and the rate of metabolism
24 (allometrically adjusted) appears to be qualitatively slower for the rat. Second, metabolism in the
25 model represents the total of all pathways, whereas it is only the minor N-nitroso metabolic route,
26 and not the oxidative route that has been proposed as a factor in RDX-induced mouse
27 carcinogenicity. Third, while blood concentration of RDX as an internal dose would be more
28 proximately relevant to the tissue than administered dose, there are no data to indicate that the
29 parent RDX is directly related to its carcinogenicity. Therefore, given the uncertainties, HEDs based
30 on both administered dose scaled by $BW^{3/4}$ and area under the curve (AUC) of RDX arterial blood
31 concentration (calculated using the PBPK model) are presented. Extrapolation based on the
32 internal dose of the parent compound is accomplished by assuming toxicological equivalence when
33 dose is expressed in terms of the AUC of the RDX blood concentration.

34 The POD estimates for rat liver carcinomas and the OSFs calculated from these PODs are
35 provided in Table D-32; detailed BMD modeling results are provided in Table D-33 (and Figure D-
36 26). Results based on two dose-metrics are presented: administered dose of RDX scaled by $BW^{3/4}$
37 (when dose is expressed in terms of mg/kg-day, this entails scaling by $BW^{-1/4}$) and AUC of RDX
38 arterial blood concentration (using PBPK modeling).

1 **Table D-32. Model predictions and oral slope factor for hepatocellular**
 2 **carcinomas in male F344 rats administered RDX in the diet for 2 years ([Levine](#)**
 3 **[et al., 1983b](#))**

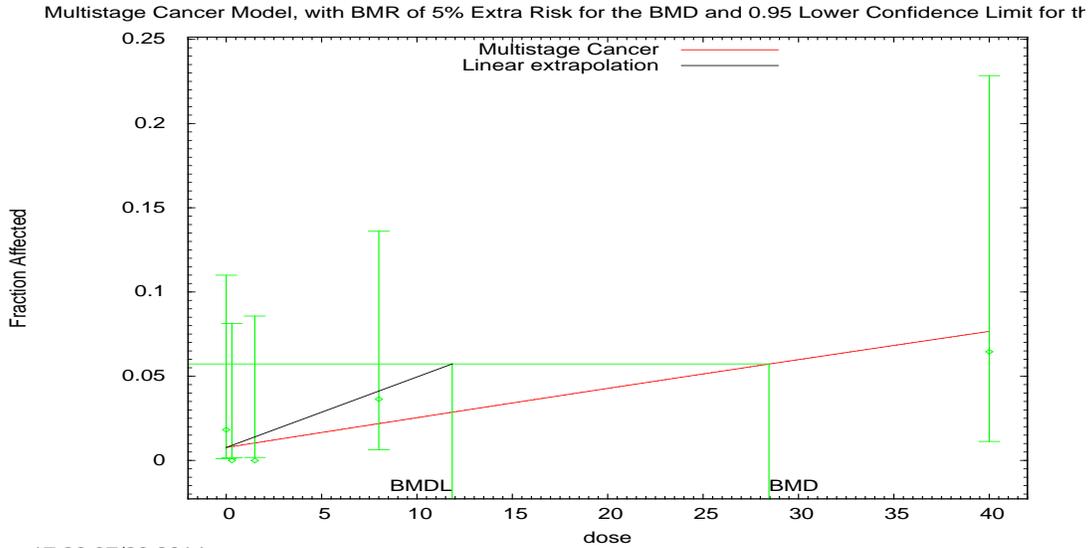
| Tumor type | Selected model | BMR | BMD, mg/kg-d | BMDL, mg/kg-d | POD = BMDL _{05-HED} , mg/kg-d | Candidate OSF ^a (mg/kg-d) ⁻¹ |
|---------------------------|----------------|-------|--------------|---------------|--|--|
| Hepatocellular carcinomas | Multistage 1° | 5% ER | 28.5 | 11.8 | 2.88 ^b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

4
 5 ^aSlope factor = BMR/BMDL_{05-HED}, where BMR = 0.05 (5% ER).
 6 ^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
 7 BW_h = 70 kg.
 8 ^cBased on toxicological equivalence of PBPK model derived AUC of RDX blood concentration.

9 **Table D-33. Model predictions for combined hepatocellular adenoma and**
 10 **carcinoma in F344 rats exposed to RDX by diet for 24 months ([Levine et al.](#)**
 11 **[1983b](#)); BMR = 5% ER**

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

12
 13 ^aSelected model in bold. Scaled residuals for the selected model for doses 0, 0.3, 1.5, 8, and 40 mg/kg-day were
 14 0.89, -0.67, -0.74, 0.74, and -0.26, respectively.
 15 ^bFor the Multistage 2°, 3°, and 4° models, the b2, b3, and b4 coefficient estimates were 0 (boundary of parameter
 16 space). The models in this row reduced to the Multistage 1° model.
 17



1
2 BMR = 5% ER; dose shown in mg/kg-day.

3 **Figure D-26. Plot of incidence rate by dose, with fitted curve for selected**
4 **model, for combined hepatocellular adenoma and carcinoma in F344 rats**
5 **exposed to RDX by diet for 24 months (Levine et al., 1983b).**

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

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

9 The parameter betas are restricted to be positive

10

11 **Benchmark Dose Computation**

12 BMR = 5% ER

13 BMD = 28.4525

14 BMDL at the 95% confidence level = 11.8487

15 BMDU at the 95% confidence level = 235.886

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

17 Multistage Cancer Slope Factor = 0.00421987

18

19 **Parameter Estimates**

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

20

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 | -21.0055 | 5 | | | |
| Fitted model | -22.5473 | 2 | 3.08372 | 3 | 0.3789 |
| Reduced model | -24.4692 | 1 | 6.92747 | 4 | 0.1398 |

2
3 AIC: = 49.0947

4
5 **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 |

6
7 Chi² = 2.4 d.f. = 3 p-value = 0.493

8

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